LIPIDS IN LACUSTRINE ENVIRONMENTS

NEIL ROBINSON

BSc

A thesis submitted to the University of Bristol in partial fulfilment of the requirements for admittance to the degree of Doctor of Philosophy.

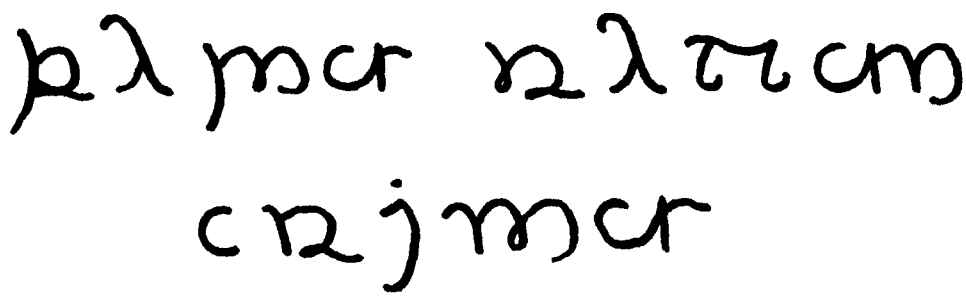
Organic Geochemistry Unit,
University of Bristol.

DECEMBER 1984

DECLARATION

I hereby declare that the work described is my own, except where otherwise stated, and this thesis has not previously been submitted for any degree.

Neil Robinson



From "The Lord cf the Rings, Vol. 1 The Fellowship of the Ring", by J.R.R.Tolkien.

To my parents, **Pam** and **Harry**,

and to **Maria Inês**

CONTENTS

PREFACE

This thesis is organised into eight chapters. Each chapter is numbered sequentially and is divided into sections, also numbered sequentially (1.1, 1.2, 1.3..... etc). Some sections are divided into subsections designated by lower-case Roman numerals (i, ii, iii.... etc). Any further divisions of these subsections are indicated by lower-case letters (a, b, c.... etc). Structures are presented in an appendix each compound being assigned an unique upper-case Roman numeral. Compounds, for which a structure is shown in the appendix, appear in the text with the appropriate Roman Numeral in parentheses following the compound name. References for the thesis as a whole follow Chapter Eight.

Chapter 1 is the general introduction providing the background to the work described in subsequent chapters detailing the aims and scope of this work. Chapters 2 to 6 report and discuss the results obtained during the course of this work. The lipids of Coniston Water sediment are described in Chapter 2, together with the lipids of an aquatic higher plant growing in the margins of the lake. Chapter 3 reports the analyses of lipids isolated from four freshwater dinoflagellates. Natural populations of organisms (rotifers, ciliated protozoa and anoxygenic phototrophic bacteria) were collected from Priest Pot. The lipids of these organisms are discussed in Chapter 4 in relation to the distribution of lipids present in the bottom sediments; changes in lipids after incorporation into the

sediments are investigated for Priest Pot by a comparison of the lipids in the surficial sediments with those in a deeper section. Chapter 5 reports the lipids present in Lake Kinneret sediment and compares them with the lipid composition determined for Peridinium cinctum (Chapter 3), the dominant member of the lake's phytoplankton. The results obtained from the study of aquatic organisms and contemporary lake sediments are extrapolated to investigate the lipid composition of Messel oil shale, an ancient lacustrine sediment, in Chapter 6. Chapter 7 provides the overall conclusions obtained from this work, comparing and contrasting the results determined for the different sediments, some suggestions for further work are given. Experimental details are provided in Chapter 8.

Some aspects of the work described in this thesis have been published: "Dinoflagellate origin for sedimentary 4α-methylsteroids and 5α(H)-stanols" (Robinson et al., 1984a) and "Lipids of aquatic organisms as potential contributors to lacustrine sediments" (Robinson et al., 1984b).

ACKNOWLEDGEMENTS

I should like to thank Professor G.Eglinton for the overall supervision of this work and for his many stimulating ideas. I gratefully acknowledge the invaluable help supplied by Dr. P.A.Cranwell of the Freshwater Biological Association, U.K., and should like to extend my thanks to the many other members of the FBA who have provided me with help in one form or another during the period of my Ph.D.

I am grateful to all the members of the OGU, both past and present, who have helped to provide a pleasant environment in which to work and with whom I have had many valuable discussions; in particular I wish to thank Drs. S.C.Brassell, S.J.Rowland and N.A.Lamb, Mr. T.Peakman, Mr. C.S.Hein and Mr. J.P.Gill. Mrs. A.P.Gowar, Miss L.Dyas and Mr. C.Saunders are acknowledged for the maintenance of the GC-MS system and other technical assistance. Special thanks go to Mrs. S.Trott and enormous admiration for her ability to remain cheerful and friendly in spite of everything.

I should like to thank Dr. R.G.Brereton for introducing word-processing into the OGU and Miss J.Stuart for assistance with computer graphics. Mrs. S.Morgan is thanked for typing the tables so quickly and accurately.

SERC are acknowledged for providing a CASE award. Professor C.Djerassi and Dr. W.C.M.C.Kokke donated standard compounds for which I am grateful.

Finally, very special thanks go to **Maria Inês Chicarelli** for so many things: valuable scientific discussion; assistance in typing this thesis and preparing the figures; financial aid when things were desperate and, especially, her love and support.

ABSTRACT

The origins and transformations of lipids in lacustrine environments have been investigated, in particular the importance of microorganisms in such processes. A dinoflagellate origin for sedimentary 4α-methylsterols is proposed based on their similarity of distribution in a natural population of the freshwater dinoflagellate Peridinium lomnickii Woloszynska collected from the waters of Priest Pot and the underlying bottom sediments. Supportive evidence for such a proposal is provided by the isolation of 4α-methylsterols from two other species of freshwater dinoflagellates, P. cinctum and Woloszynskia coronata, and the presence of relatively abundant levels of 4α-methylsterols in the sediments of Lake Kinneret, to which P. cinctum is a major contributor. The presence of 5α(H)-cholestan-3β-ol as a major sterol of P. lomnickii demonstrates that sedimentary 5α(H)-stanols can in part originate by direct input from dinoflagellates. 4α-Methylsteroidal ketones have been isolated from P. lomnickii and W.coronata. Such compounds, other than dinosterone (4α,23,24-trimethyl-5α(H)-cholest-22-en-3-one), have not previously been reported to occur in organisms. An input of 4α-methylsteroidal ketones from P. lomnickii to the sediments of Priest Pot was inferred from the similarity of distribution of 4α-methylsteroidal ketones isolated from both. Gorgosterol (22,23-methylene-23,24-dimethyl-cholest-5-en-3β-ol) and gorgostanol, not previously reported to

occur in freshwater organisms, were isolated from the dinoflagellate Ceratium hirundinella. Peridinosterol (4α,23,24-trimethyl-5α(H)-cholest-17(20)-en-3β-ol) has been isolated from the sediments of Lake Kinneret. The range and distribution of sterols and steroidal ketones present in the four freshwater dinoflagellates studied, provides new information on the biosynthesis of such compounds.

Natural populations of rotifers, ciliated protozoa and anoxygenic phototrophic bacteria, present at specific horizons in the water column of Priest Pot, were obtained during summer stratification when the hypolimnion was anoxic. The lipids of these organisms have been compared with those of the underlying bottom sediments. Algal $\Delta^{5,7}$-sterols were removed within the water column and were not incorporated into the sediments, such removal is proposed to arise from editing by zooplankton feeding. Δ^{7}-sterols, originating from Chlorophyceae, were resistant to degradation and were preserved in the bottom sediments.

Comparison of the lipids isolated from different depth sections of the sediments of Coniston Water, Priest Pot and Lake Kinneret, demonstrated that shorter chain homologues and unsaturated compounds were preferentially removed. Preservation of labile compounds is better in the sediments of Priest Pot and Lake Kinneret, the productivity and bathymetry of which produce anoxic sediments and seasonally anoxic hypolimnia, than in the sediments of the oligo-mesotrophic Coniston Water. The ratio of allochthonous : autochthonous lipids in the sediments of these lakes, reflects their trophic status and the vegetation of their

catchment areas. Bound lipids are stabilised relative to free, and show a relatively greater content of bacterially-derived material.

Analysis of the free lipids of a sample of the 50 x 10^6 years old lacustrine Messel oil shale revealed many similarities with the sediments of contemporary lakes. Dinoflagellate activity at the time of deposition was inferred from the distribution of 4α-methylsterols and 4α-methylsteroidal ketones isolated from the shale. A relatively small higher plant input, but a large bacterial input, is proposed.

GLOSSARY

ai : anteiso

amu : atomic mass units

Bound : lipids released by acidic hydrolysis of pre-extracted sediment

BSTFA : bis(trimethylsilyl)trifluoroacetamide

CPI : carbon preference index

DSDP : Deep Sea Drilling Project

FAME : fatty acid methyl ester(s)

Free : lipids released by solvent extraction of sediment without an hydrolysis step

GC : gas chromatography, gas chromatogram, gas chromatographic

HPLC : high performance liquid chromatography

i : iso

$M^{\cdot+}$: molecular ion

MS : mass spectroscopy, mass spectrum, mass spectrometric

nmr : nuclear magnetic resonance

TLC : thin layer chromatography

TMS : trimethylsilyl

CHAPTER ONE

GENERAL INTRODUCTION

1.1 General

Organic geochemistry involves the study of the distribution and fate of carbon and its compounds in the biosphere and lithosphere. For living organisms the oceans and the atmosphere represent the most important reservoirs of carbon, in the form of carbon dioxide CO_3^{2-} or HCO_3^-. The amount of carbon in these two reservoirs, however, is only a very small fraction of the Earth's total stock of carbon (Fig. 1.1/1). In the global environment, carbon in the form of organic matter is mainly recycled. Plants produce organic compounds from carbon dioxide during photosynthesis; decomposition of the primary products occurs when used as a food source by animals or during natural decay by microorganisms.

Some material, however, escapes recycling and becomes incorporated into the sediments. Organic matter thus incorporated contains information relating to its origin and history. One aim of organic geochemistry is to unlock this information. There are, however, many variables which complicate the picture; biolipids produced by living organisms and incorporated into sediments may then undergo various biological and chemical processes, which can modify them and produce the geolipids found in sediments. In aquatic environments, biologically mediated transformations of biolipids proceed in the water column during sedimentation and continue in the surficial sediments (Fig. 1.1/2). These biological transformations occur by bacterial attack and feeding processes, where, for example, certain algal lipids may be removed or modified during passage

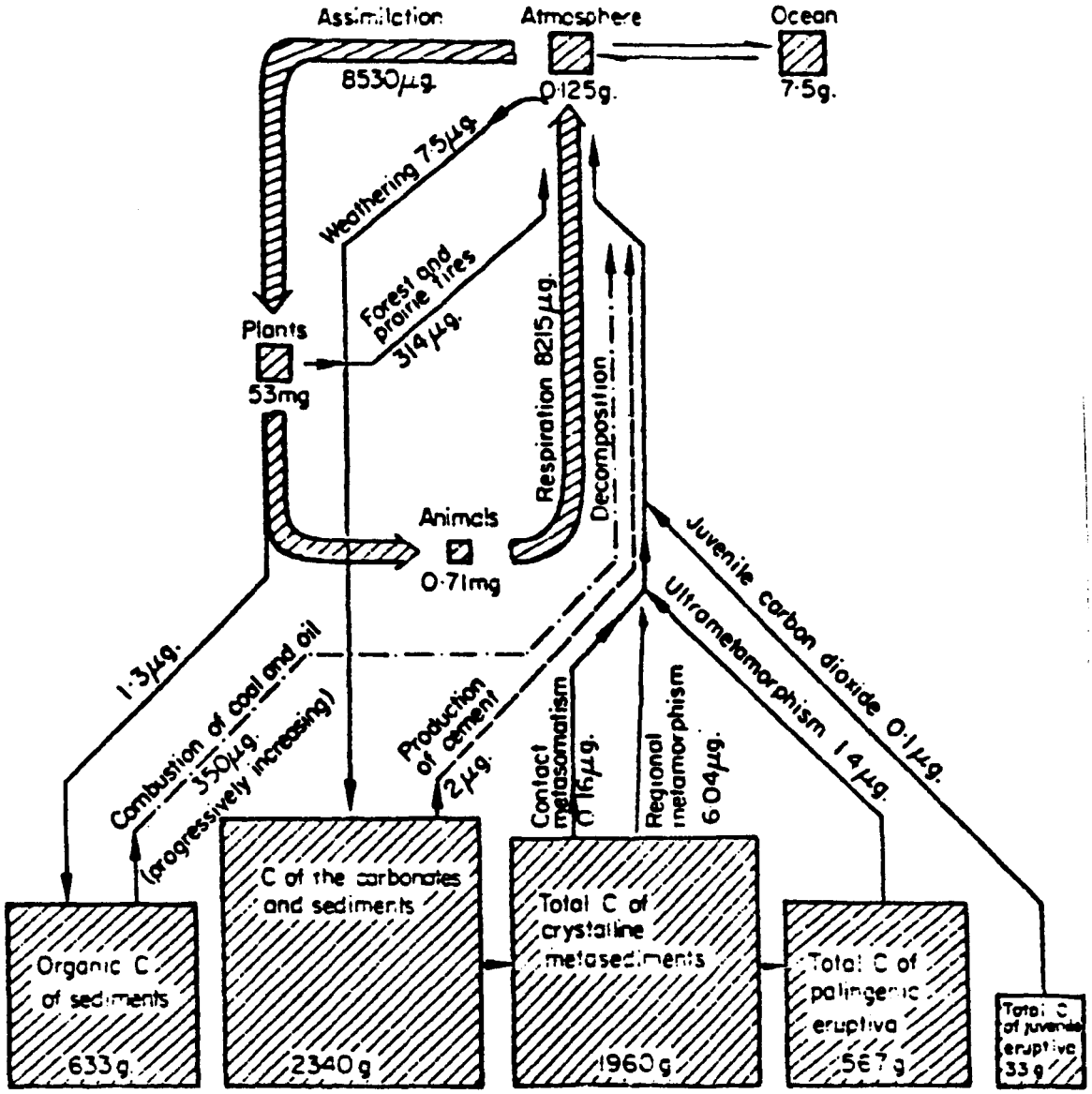


Fig. 1.1/1. Geochemical carbon cycle in Nature showing relative weights of carbon in the various reservoirs and the transport routes. (Reproduced from Riley and Chester, 1977) The majority of the Earth's stock of carbon is present in sediments. Carbon is released to the atmosphere, where it may be utilised by organisms, by volcanic activity, metamorphic changes in rocks within the Earth's crust, brought about by the agencies of heat, pressure and chemically active fluids, and by anthropogenic combustion of coal and oil, a process which is progressively increasing.

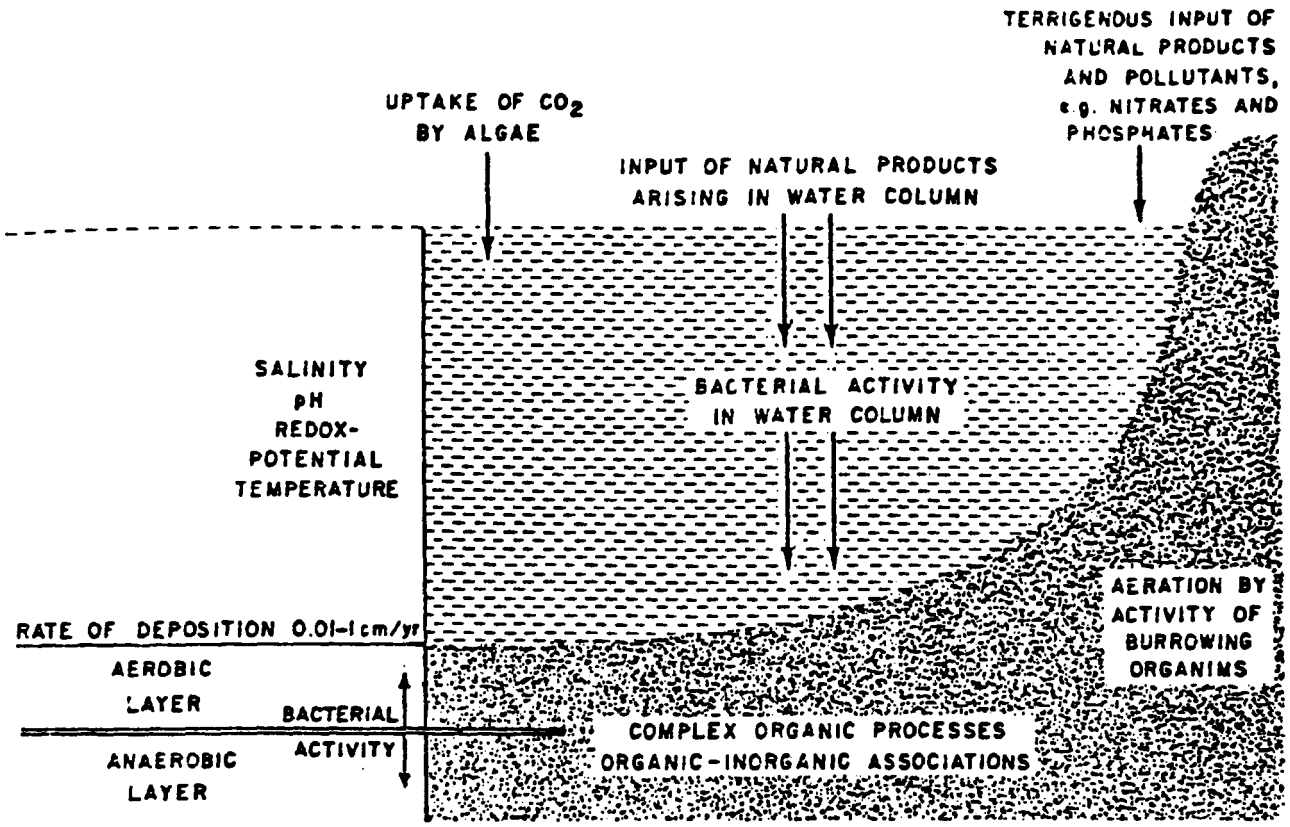


Fig. 1.1/2. Schematic diagram showing the various interdependent chemical and biological factors which effect the ultimate fate of biolipids deposited in Recent sediments. (Reproduced from Philp et al., 1976)

through the gut of zooplankton (Eglinton et al., 1979; Prahl et al., 1984).

Sediments formed from deposition within aquatic environments contain large amounts of water, living microorganisms, dead organic matter and minerals, arising from various sedimentary processes. The resulting mixture is out of equilibrium and, as a particular sediment layer becomes compacted and buried beneath overlying, subsequently deposited layers, it experiences diagenesis, a process whereby the system tends to approach equilibrium and the sediment becomes consolidated. Diagenesis takes place under conditions of shallow burial, the depth order is normally of the order of a few hundred metres, the increase of temperature and pressure is small, and transformations occur under mild conditions. During early diagenesis, one of the agents of transformation is microbial activity. The microbial organisms present effect transformations within the sedimentary lipids by reworking lipids deposited from the water column and by contributing their own lipids to the sedimentary lipid assemblage. This transformation process will be referred to as lipid diagenesis and tends to produce lipids in a more stable form, by defunctionalisation and isomerisation. At present there is no suitable term for the transformations that affect lipids during sedimentation through the water column. The end of diagenesis may be placed at the level where extractable humic acids have decreased to a minor amount, and where most carboxyl groups have been removed (Tissot and Welte, 1978). With increasing burial depth the sediment experiences increasing pressures and temperatures, which produce further changes in the

lipid composition and may result in oil generation within the zone of catagenesis.

Thus, when compared with the biolipid precursor,structures of geolipids in sediments may be (1) unaltered, (2) partly altered, rearranged or degraded, (3) extensively altered,e.g. after thermal treatment, or (4) completely degraded to carbon dioxide or methane. Molecular organic geochemical analyis of aquatic sediments aims at (1) the recognition of lipid patterns characteristic of different environments, (2) the recognition of intermediates in the pathways of conversion of biolipids into geolipids and (3) recognition of specific biolipids unique to one organism or class of organisms, which give rise to geolipids that may be used as biological marker compounds for input from those organisms. Some of the sedimentary compounds encountered have not been characterised previously by natural product chemists in living organisms and many organisms acting as sources of sedimentary lipids have never been studied.

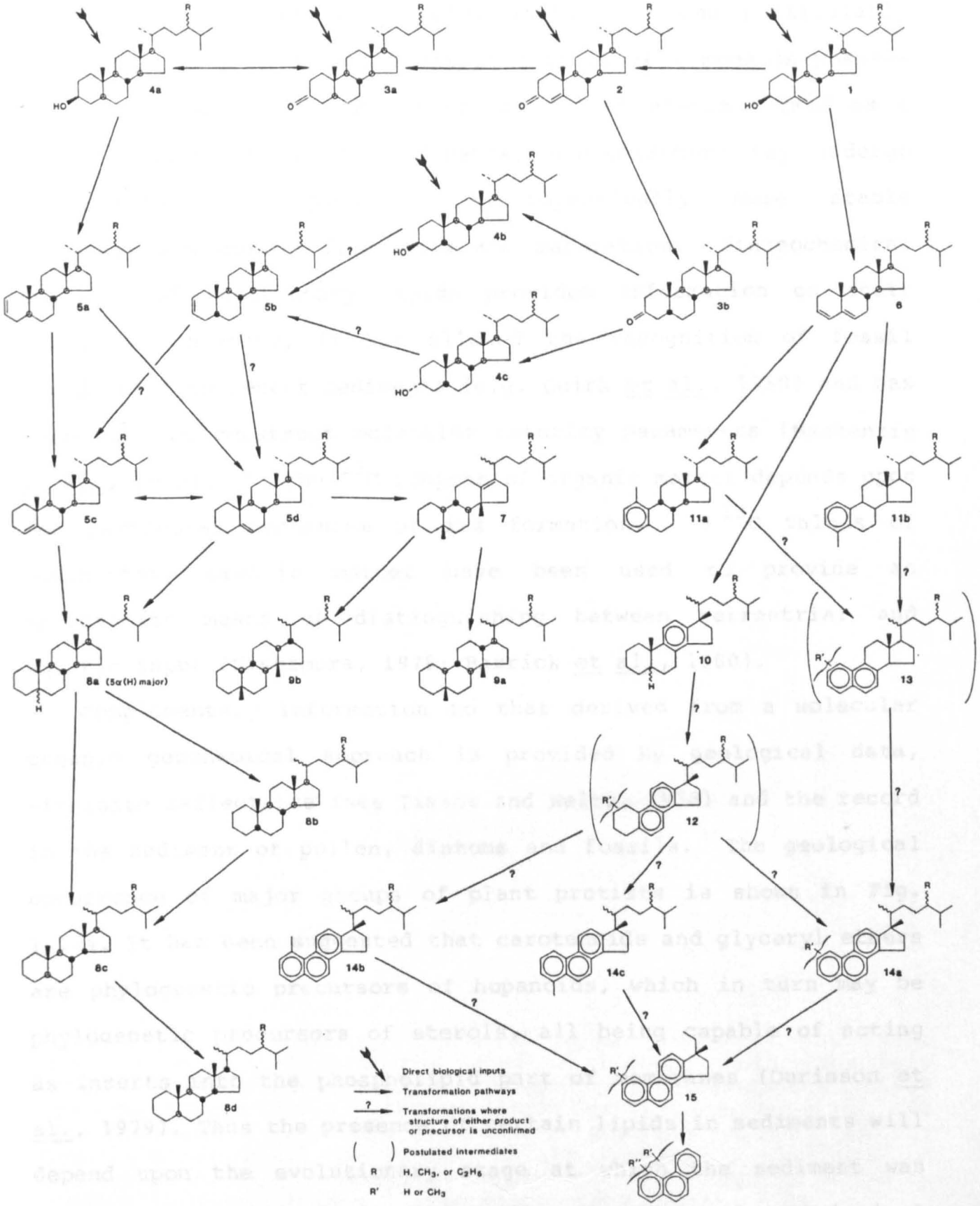
Examples of sedimentary lipids present in a state unaltered from their source organisms include sterols (e.g. Mclean et al., 1958; Schwendinger and Erdman, 1964; Boon et al., 1979; Robinson et al., 1984), fatty acids (e.g. Parker, 1969; Cranwell, 1974; Volkman and Johns, 1977) and triacylglycerols (Boon et al., 1980; Wakeham et al., 1980). Sedimentary lipids, which, although partly altered, may still be recognised as arising from well known natural products include petroporphyrins, originating from chlorophylls (e.g. Quirke and Maxwell, 1980; Fookes, 1983; Chicarelli and Maxwell, 1984) and steroidal hydrocarbons, originating from sterols (Mackenzie et al., 1982 and references therein). Some compounds may originate in part from direct biological input and

partly from transformation from other compounds, e.g. 5α(H)-stanols have been shown to originate from dinoflagellates (Robinson et al., 1984) or are formed by microbial hydrogenation of stenols (Gaskell and Eglinton, 1975). The complexity of possible transformation pathways in sediments is illustrated for steroids in Fig. 1.1/3.

The majority of sedimentary compounds are members of the two major biogenetic classes, polyacetate-derived compounds and mevalonate-derived compounds. Typical examples of polyacetate-derived compounds include the straight chain fatty acids, ketones, alcohols and hydrocarbons, and the corresponding iso- and anteiso-branched chain series. Examples of mevalonate-derived compounds include sesqui-, di-, tri- and tetraterpenes in the acyclic series, and steroids and triterpenoids in the cyclic series. There are several parameters useful in molecular organic geochemistry, consequent upon the biosynthetic origin of the sedimentary organic matter. (1) Homologous series of compounds, biosynthesised by the polyacetate route, are built up from C_2 units and so often show an odd or even carbon number preference, a feature which may be preserved in sediments. (2) The carbon number range of such lipid series varies with the source organism. In general algae and other aquatic microorganisms synthesise shorter-chain homologous series than those in the wax coatings of higher plants, allowing, in many cases, inputs from autochthonous and allochthonous sources to be distinguished. (3) Compounds biosynthesised by the mevalonate route may form pseudohomologous series, such as acyclic isoprenoid alkanes or sterols, different members of which

Fig. 1.1/3 (OVER). Schematic summary of proposed pathways of sterol diagenesis and catagenesis. (Reproduced from Mackenzie et al., 1982) The scheme does not include 4-methylsteroids, also a number of recent findings render it incomplete: compounds of general structure 5d can undergo transformation to produce a series of spirosterenes (Peakman et al., 1984), although spirosterenes may also originate from $\Delta^{8(14)}$-sterols (Peakman, personal communication); stera-3,5-dienes (6) may undergo acid catalysed rearrangement to produce B ring aromatic anthrasteroids (Hussler and Albrecht, 1983; Hoffmann, 1984) which may be further aromatised (Hoffmann, 1984); 5β(H)-A-nor-steranes, identified in sediments, have been proposed to originate from Δ^{5}-sterols (1) via sponge dietary processes (Van Graas et al., 1982a), alternatively 5β(H)-A-nor-steroidal ketones may be the precursors of the 5β(H)-A-nor-steranes (McEvoy, 1983); ring A degraded steroids, including ketones (Van Graas et al., 1982b), aromatics and diasterenes (Peakman, Farrimond, Brassell, Eglinton and Maxwell, unpublished results), have been identified in sediments.

Fig. 1.1/3.


4a
3a
2
1
4b
5a
5b
3b
6
4c
5c
5d
7
11a
11b
8a (5α(H) major)
9b
9a
10
13
8b
12
8c
14b
14c
14a
8d
15
16
Direct biological inputs
Transformation pathways
Transformations where structure of either product or precursor is unconfirmed
Postulated intermediates
R H, CH3, or C2H5
R' H or CH3
R'' H, CH3, C2H5, or C3H7

may be indicative of inputs from different organisms (Brassell et al., 1981; Huang and Meinschein, 1979). (4) One particularly important aspect of biosynthesis is that where compounds possess one or more chiral centres they are almost always formed as a single stereoisomer. In sediments, stereoisomers may undergo epimerisation to give a thermodynamically more stable configuration during diagenesis and maturation. Stereochemical analysis of sedimentary lipids provides information on their origin and history, it has allowed the recognition of fossil lipid input to recent sediments (e.g. Quirk et al., 1980) and has been used to construct molecular maturity parameters (Mackenzie et al., 1980). (5) The ^{13}C content of organic matter depends upon the particular mechanism of its formation. $\delta\,^{13}C$ values of sedimentary organic matter have been used to provide an approximate means of distinguishing between terrestrial and aquatic input (Nishimura, 1978; Barrick et al., 1980).

Complementary information to that derived from a molecular organic geochemical approach is provided by geological data, vitrinite reflectance (see Tissot and Welte, 1978) and the record in the sediment of pollen, diatoms and fossils. The geological occurrence of major groups of plant protists is shown in Fig. 1.1/4. It has been suggested that carotenoids and glyceryl ethers are phylogenetic precursors of hopanoids, which in turn may be phylogenetic precursors of sterols, all being capable of acting as inserts into the phospholipid part of membranes (Ourisson et al., 1979). Thus the presence of certain lipids in sediments will depend upon the evolutionary stage at which the sediment was deposited and should correlate with the micropalaeontological

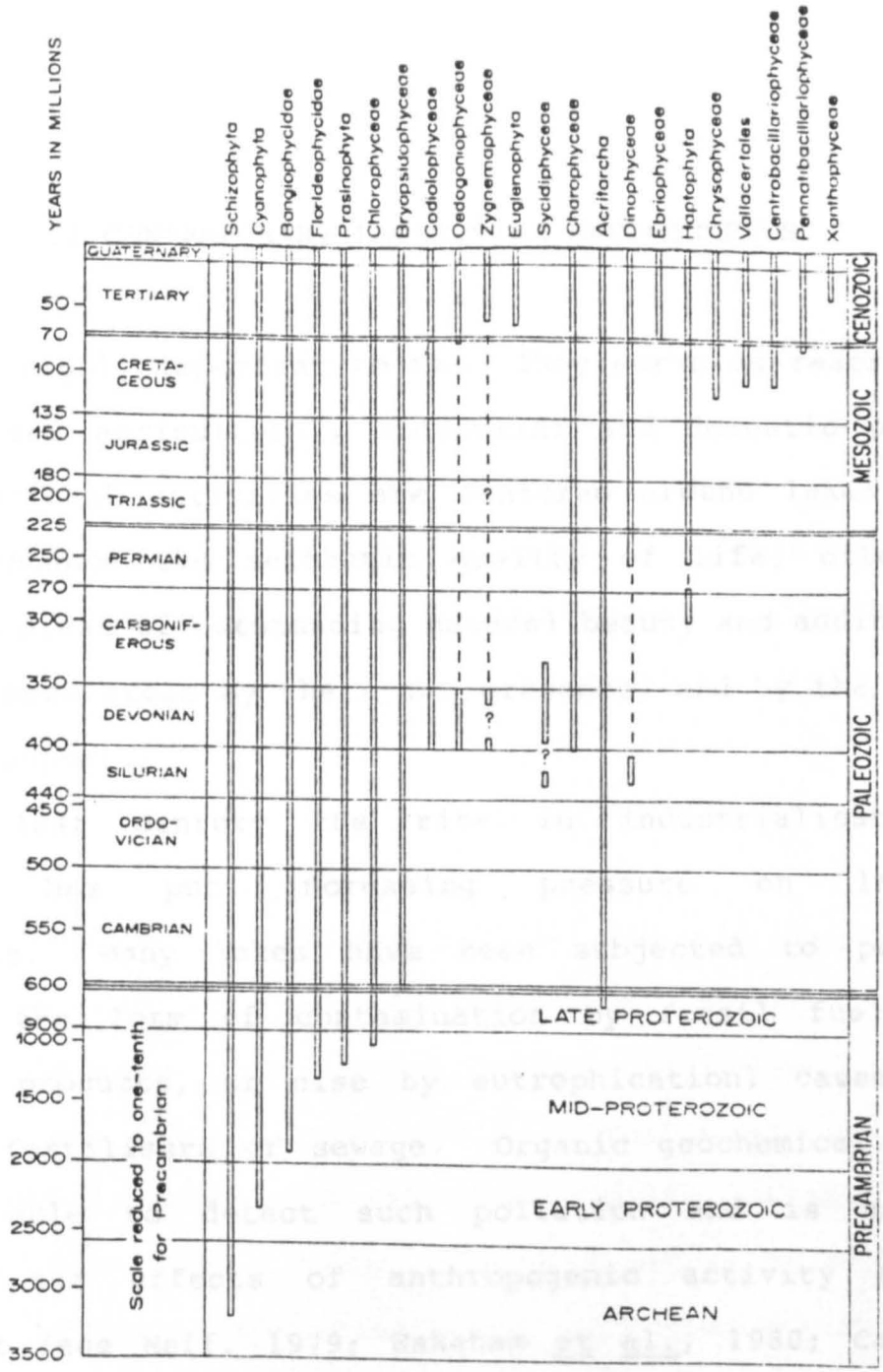


Fig. 1.1/4. Geologic occurrence of major groups of the plant protists, mostly shown to the level of classes. Both the extent of the geologic record and the diversity of organisms involved are demonstrated, e.g. the earliest fossils attributed to the Dinophyceae are from the Silurian, a gap then appears in the fossil record until the middle of the Permian. The absence of fossils does not necessarily imply that the organisms which produce them were absent. (Reproduced from Tappan, 1980)

record.

1.2 CONTEMPORARY LACUSTRINE ENVIRONMENTS

Lakes are highly important to Man. They serve as reservoirs of freshwater for agricultural, industrial and domestic purposes. Many recreational activities are centered around lakes. Lakes can also enhance the aesthetic quality of life, often being situated in areas of outstanding natural beauty and adding to the beauty of these areas by their own presence, and by the wildlife that they support.

In the last century the rise in industrialisation and population has put increasing pressure on lacustrine environments. Many lakes have been subjected to pollution, either in the form of contamination by fossil fuel or its combustion products, or else by eutrophication, caused by an influx of fertilisers or sewage. Organic geochemical analysis has been able to detect such pollution and is useful in monitoring the effects of anthropogenic activity upon the environment (see Neff, 1979; Wakeham et al., 1980; Cardoso et al., 1983).

Lakes may have been formed in a variety of ways. Tectonic mechanisms have been responsible for very large-scale movements, which in late Miocene times caused the formation of vast inland seas in south east Europe and southern Asia, remnants of which include the Caspian and Black Seas. In East Africa a smaller crustal sag accommodates Lake Victoria and examples of rift

valley lakes include Tahoe (California), Baikal (USSR), Tanganyika and Malawi (East Africa), and the Dead Sea. Lakes of volcanic origin are often deep and usually smaller than those of tectonic origin, e.g., Crater Lake, Oregon. Many lakes of high latitude areas have been formed by the effects of glaciation, including ice damming, drainage barriers formed of moraine materials, freeze-thaw effects and valley glaciation, which produces long, narrow, deep lakes such as fjords. Fluvial action may produce lakes, as may aeolian transport of sediments.

The small size of most lakes means that there is a large ratio between land drainage and lake area, resulting in much higher sedimentation rates than in oceanic environments. Consequently, most lakes are transitory features on a geological time scale.

Most lakes are well flushed and the chemical constituents of their waters do not accumulate beyond the potable range, resulting in much lower concentrations of salts, carbonate, bicarbonate and sulphate than found in the marine environment. Under certain circumstances saline or hypersaline lakes, such as Solar Lake, Elat, may develop. The differences in chemistry of fresh and saline water result in differences of microbial populations and, hence, can result in differences in sedimentary lipid composition. The morphometry of a lake can play a large part in affecting its chemistry, determining, for instance, whether or not a seasonal pycnocline develops.

Lacustrine environments are suitable sites to study for organic geochemical research. Small lakes situated in glacially-formed valleys receive a more homogeneous allochthonous

organic sediment input, derived from soils eroded from the drainage basin, than does the coastal marine environment which receives sediment by potamic transport from a large land area and in which tidal movements cause resuspension and mixing. The enclosed nature of lakes also makes it easier to define the autochthonous inputs. Lacustrine environments thus constitute simpler ecosystems than marine ones. Generally lakes are more productive than open sea areas, resulting in greater sedimentary organic carbon contents. Complementary information on inputs to lakes is provided by the sedimentary pollen and diatom record (e.g. Pennington et al., 1972; Haworth, 1984). Information gained from investigations carried out in lakes can often be successfully employed in marine studies.

Some of the lakes studied by organic geochemists are listed in Table 1.2/1. The organic geochemistry literature has recently been well reviewed by Cranwell (1982) and the reader is directed to this and other similar articles for a fuller compilation of published work in organic geochemistry (e.g. Philp et al., 1976; Barnes and Barnes, 1978; Simoneit, 1978; Mackenzie et al., 1982; Brassell et al., 1983). Some, however, of the main findings, pertinent to this thesis, will be discussed and although directed towards lacustrine environments are normally valid in context to other types of aquatic environments.

As stated previously there are various parameters useful to molecular organic geochemical studies. Leaf epicuticular waxes have been shown to contain lipids, having a high CPI, in the range C_{27} to C_{33} (Eglinton and Hunneman, 1967; Tulloch, 1976). The presence of similar distributions of lipids in sediments have

Table 1.2/1. Examples of contemporary lakes studied by organic geochemists

Lake (Trophic status[a])	Studies Made	Reference
Cam Loch, Sutherland, U.K.	Hydrocarbons, ketones, alcohols and fatty acids.	Cranwell, 1977
(Oligotrophic, Mesotrophic)	Branched/cyclic alkanols	Cranwell, 1980
Grasmere, Cumbria, U.K.	Straight-chain alkanes and alkanoic acids.	Brooks et al., 1976
(Oligotrophic)	Branched/cyclic alkanes and alkanoic acids.	Brooks et al., 1977
Lake Biwa, Japan.	Alkanes	Ishiwatari, 1976
(Oligotrophic, mesotrophic)	Fatty acids and perylene	Ishiwatari, 1976
	Sterols	Ogura, 1976
	Fatty acids	Ishiwatari and Kawamura, 1977
Loch Clair, Sutherland, U.K.	Branched/cyclic alkanols	Cranwell, 1980
(Oligotrophic)	Hydrocarbons, ketones, alcohols and fatty acids; diagenesis of free and bound lipids	Cranwell, 1981a
	Alkyl and steryl esters	Cranwell and Volkman, 1981
Lake Haruna, Japan.	Fatty acids	Ishiwatari et al., 1977
(Oligotrophic, mesotrophic)	Alkanes	Ishiwatari et al., 1980
Lake Huron, U.S.A. (Oligotrophic)	Hydrocarbons and fatty acids	Meyers and Takeuchi, 1979
Pyramid Lake, U.S.A. (Oligotrophic, mesotrophic)	Hydrocarbons and fatty acids	Meyers et al., 1980
Crose Mere, Cheshire, U.K. (Eutrophic)	Branched/cyclic alkanols	Cranwell, 1980
Esthwaite Water, Cumbria, U.K. (Eutrophic)	Straight-chain and alkanoic acids	Brooks et al., 1976
	Branched/cyclic alkanes and alkanoic acids	Brooks et al., 1977
Greifensee, Switzerland (Oligotrphic, mesotrophic, eutrophic)	Hydrocarbons	Giger et al., 1980
Rostherne Mere, Cheshire, U.K.	Straight-chain hydrocarbons and fatty acids	Brooks et al., 1976
(Euthrophic)	Branched/cyclic hydrocarbons and fatty acids	Brooks et al., 1977
	Sterols	Gaskell and Eglinton, 1976
	Hydrocarbons and fatty acids	Cardoso et al. 1983

(a) Where more than one trophic state is given this indicates a change in the lake from the time of deposition of one sediment section to another.

been attributed to higher plant sources (e.g. Cranwell, 1973; 1974; Brooks et al., 1976; Cardoso et al., 1983). Similarly, the characteristic carbon number range of algal lipids, smaller than that of the corresponding higher plant lipids, has been used as a marker of algal input to sediments. The n-alkanoic acids of algae are often maximal at C_{16} with significant C_{14} and C_{18} components (Erwin, 1973) and the presence of these in sediments has been used as a marker of autochthonous production at the time of deposition, in a number of lakes (e.g. Cranwell, 1974; Brooks et al., 1976; Ishiwatari and Kawamura, 1977; Meyers et al., 1980). Care must be taken, however, not to confuse dominantly autochthonous input with the effect of lipid diagenesis preferentially degrading shorter chain homologues. This will be discussed further later. The proportion of n-heptadecane amongst the sedimentary alkanes has also been widely used to estimate trophic level at the time of deposition, being the dominant hydrocarbon of many algae and bacteria (Han et al., 1968; Han and Calvin, 1969; Gelpi et al., 1970).

Sterols form a significant fraction of the surficial sedimentary lipids of the most lakes, being present in eucaryotes, where they are assumed to act as rigidifiers of biomembranes. Sedimentary sterols are commonly found in the range C_{27} to C_{29}; the proportions of the C_{27} and C_{29} components have been proposed as a means of defining ecological systems (Huang and Meinschein, 1979). It is now recognised, however, that in many productive environments, where there is an input of sterols from a range of organisms, a triangular plot of C_{27}, C_{28} and C_{29} sterols as proposed by Huang and Meinschein (1979) does

not reflect the sources of the sedimentary sterols. Such a plot does not have any provision for 4-methylsterols and it places 24-ethylsterols and 23,24-dimethylsterols together as being indicative of higher plant input, whereas the latter have been found in dinoflagellates (Withers et al., 1982; Nichols et al., 1984; Chapter 3, this thesis) and diatoms (Volkman et al., 1980). Dinosterol (4α,23,24-trimethyl-5α(H)-cholest-22E-en-3β-ol), first recognised in the dinoflagellate Gonyaulax tamarensis (Shimizu et al., 1976), has been used as a biological marker for dinoflagellate contributions to marine sediments (Boon et al., 1979; Gagosian et al., 1980; De Leeuw et al., 1983; Brassell and Eglinton, 1983). In this thesis dinosterol and 4α-methylsterols in general are shown to be markers of dinoflagellate input to freshwater sediments.

Lipids characteristic of bacteria occur widely in sediments, for example iso- and anteiso- branched C_{15} and C_{17} acids are widely used as a marker for bacterial input to sediments (Leo and Parker, 1966; Parker, 1969; Cranwell, 1974, 1978, 1981a; Cardoso et al., 1983). Certain double bond positional isomers of monounsaturated fatty acids have been used as specific bacterial markers in marine sediments (Gillan et al., 1983) and in detritus resulting from microbial attack on freshwater algae (Cranwell, 1979).

Extraction procedures involving saponification, transesterification or demineralisation were formerly used in the isolation of sedimentary lipids, but it is now recognised that sedimentary lipids can exist in more than one chemically-distinct form and that maximum information can only be obtained if the

extraction procedure allows these to be analysed separately (Farrington and Quinn, 1971, 1973; Nishimura, 1977; Cranwell, 1978). Lipids isolated by direct solvent extraction will hereafter be referred to as free lipids, whilst those isolated from previously extracted sediment following hydrolysis, will be referred to as bound lipids. The exact binding mechanism for bound lipids is uncertain; polar molecules, such as carboxylic acids, may occur as salts and complexes with the metal ions of clay minerals or esterified to hydroxyl groups which are part of the kerogen or humic polymeric insoluble organic matter. Non-polar lipids, such as hydrocarbons, lacking functional groups, also occur in the bound fraction, suggesting that another binding mechanism also operates, possibly involving trapping in macromolecular cage structures formed by hydrogen bonding of peripheral groups or by release from polymeric cell wall material, broken down during hydrolysis. The last is supported by the recognition of a greater microbial contribution to bound lipids than to free (Brooks et al., 1976; Cranwell, 1978, 1979; 1981a; Cardoso et al., 1983). Stereochemical analysis has been used to differentiate the sources of free and bound 3-hydroxy acids in an oligotrophic lake (Cranwell, 1981b), bound 3-hydroxy acids being attributed to a microbial cell wall origin. Recently, bound 3-hydroxy acids present in a Namibian Shelf sediment sample were shown to occur with an amide linkage and cooccurred with monosaccharides specific for carbohydrates associated with bacterial cell walls, providing strong evidence for a bacterial lipopolysaccharide origin of the bound 3-hydroxy acids (Klok, 1984).

Sedimentary lipids may have been transformed or degraded from their original state in source organisms. Lipid diagenesis can take place during sedimentation through the water column, or, for terrestrially derived lipids, before transference to the aquatic environment (Quirk, 1978). The degree of sedimentary lipid preservation is affected by the environment under which deposition takes place. An oxic water column and sediment/water interface results in poor preservation of organic matter, both quantitatively and at the molecular level (Didyk et al., 1978), whereas anoxicity within the water column aids preservation by decreasing predation and bacterial degradation. As bacteria are concentrated at the sediment/water interface of both oxic and anoxic sediments, rapid removal of organic matter from this active zone will enhance the degree of lipid preservation. Anoxic basins are, generally, the result of high productivity and so are accompanied by high sedimentation rates, providing a further factor in the greater degree of lipid preservation in anoxic compared with oxic aquatic environments. A high sedimentation rate, due to extensive land erosion, has been used to explain the good preservation of lipids in the sediment of an oligotrophic lake (Meyers et al., 1984).

The chemical state of lipids plays a major role in determining their preservation; generally, unsaturated compounds are removed faster than saturated molecules (Kawamura et al., 1980; Cranwell, 1981a; Cardoso et al., 1983) and shorter chain lipids suffer a greater degradation than corresponding longer chain homologues (Quirk, 1978; Cranwell, 1981a; Cardoso et al., 1983).

A variety of methods have been employed to investigate the initial stages of lipid diagenesis, including 1) following changes in the abundance of major constituents in the sediment profile (Giger et al., 1980); 2) simplified laboratory studies of the marine food web, in which the lipid content of zooplankton faecal pellets are compared with that of the phytoplankton upon which they are feeding (Eglinton et al., 1979; Prahl et al., 1983); 3) the deployment of sediment traps at varying depths in the marine environment to determine the fluxes of lipids in sedimentary particulates (Wakeham et al., 1980) and 4) incubation of radio-labelled marker compounds under environmental conditions (Rhead et al., 1971; Javor et al., 1979; Edmunds et al., 1980).

1.3 ANCIENT LACUSTRINE ENVIRONMENTS

Sedimentary deposits forming in shallow, productive lakes in which autochthonous input is dominant, are believed to be modern analogues of deposits which have produced certain oil shales (Bradley, 1966), while sediments of unproductive lakes, in which organic input is mainly of higher plant, peat forming origin, may be modern equivalents of deposition which formed certain coals (Cooper and Murchison, 1969). Lacustrine oil shales include the very large Green River formation shales of Eocene age in Colorado, Utah and Wyoming (up to 600m thick), the Triassic beds in the Stanleyville basin, Zaire and the Albert Shales of Mississippian age, New Brunswick, Canada. Smaller organic-rich

lacustrine oil shales include the Eocene Messel oil shale, W. Germany. Extraction of oil from oil shales involves pyrolysis of the rock and, although the industry reached its highest point of development immediately after World War II, new processes are continually being developed. The lacustrine Green River shales constitute the biggest reserve of oil shale oil in the world, estimated to be ca. 300 billion tons of potential oil. An approximate estimation of the main oil shale reserves in the world is given in Table 1.3/1. Important coal occurrences developed around huge freshwater lacustrine basins such as the carboniferous coals of Bohemia, the Saar district in Germany, the Massif Central in France, and Spain (Tissot and Welte, 1978).

Geochemical studies of ancient lacustrine sediments have revealed them to contain similar suites of lipids as contemporary lacustrine environments (Gallegos, 1971; Kimble et al., 1974; Philp et al., 1976; Habermehl and Hundrieser, 1983).

Some of the lipids present in ancient lacustrine sediments are unchanged from when they where deposited, giving direct information on the source organisms contributing to the sediment; other lipids found in ancient lacustrine sediments are absent from contemporary sediments, but, using a carbon skeleton approach (Eglinton and Calvin, 1967) and a refinement of this to include stereochemical considerations (Maxwell et al., 1972), presumed precursor biolipid-geolipid relationships may be determined. Thus, for instance, the sterols present in contemporary sediments and derived from organisms may undergo a series of diagenetic transformations within the sediment, forming the sterenes, steranes, diasterenes, diasteranes and steroidal

Table 1.3/1. Approximate estimation of the main oil shale reserves.(Reproduced from Tissot and Welte)

North America		357 000
USA	350 000	
Canada	7 000	
South America:Brazil		127 000
Northern and Western Europe		1 000
Italy		5 600
USSR (including Siberia)		18 000
Zaire		16 000
China		4 400
Other countries		ca. 1 000
	Total	ca. 530 000

million m^3 potential oil.

aromatic hydrocarbons found in ancient sediments and petroleums (Mackenzie et al., 1982). A sediment reaches maturity when it enters the principle zone of oil generation. Measurements of maturity can be based on reflectance values, physical state of the kerogen or analysis of the bitumen, maturity being obtained when the chemical constituents of the sediment are present at their thermodynamic ratios. The extent to which geolipids in an ancient sediment have been transformed from their original biolipids, is, to a large extent, a reflection of the burial temperature history of that sediment.

Interpretation of the lipid distributions present has allowed aspects of the original paleoenvironments of deposition of ancient sediments to be assessed and the diagenetic/maturation histories of different oil shales to be compared (Kimble et al., 1974).

1.4 THIS WORK

The work described in this thesis has as a unifying theme the important role that microorganisms play in determining the distribution of lipids in lacustrine environments. The origins of the lipids in relation to inputs and diagenetic transformations will be investigated and discussed. Thus, emphasis will be placed upon correlations between lipid distributions in known source organisms and sediments, and on the role of microbial organisms in producing rapid rates of lipid

transformations within the water column and surface sediment compared with the slow physico-chemical alterations that take place with increasing burial depth.

Chapter 2 reports the results of an investigation into the lipids of the surface sediments of Coniston Water, a mesotrophic lake situated in the English Lake District. The aims of this investigation were to 1) study the early stages of lipid diagenesis for a number of compound classes in such a lake; 2) to compare the sedimentary lipid signature with the lipid composition of known sources, 3) to estimate the relative importance of the known sources in contributing to various compound classes and 4) to determine some of the factors affecting differences between free and bound lipid distributions.

Chapter 3 reports a study of the extractable lipids of four freshwater species of dinoflagellates. The aims of this study were to 1) extend the knowledge of the lipid composition of sources of organic matter to lacustrine sediments; 2) to compare the lipid composition of freshwater dinoflagellates with that of marine species; 3) to gain an insight into biosynthesis and chemical state of lipids in dinoflagellates and 4) to find which dinoflagellate biolipids would give rise to geolipid marker compounds specific for dinoflagellate inputs to lacustrine sediments. The results of this study are used to recognise dinoflagellate derived lipids in three very different lacustrine sediments.

Chapter 4 describes an investigation into the lipids present in Priest Pot, a small, highly productive lake situated in the

English Lake District. A characteristic of this lake is summer stratification of the water column leading to vertical stratification of the water column organisms, and this phenomenon made possible the collection of three different natural populations of organisms (rotifers, ciliated protozoa and Chlorobium bacteria) in >90% purity, for analysis of their lipid composition. The aims of the investigation were to 1) follow the fate and distribution of lipids down a vertical profile of the water column; 2) to relate such changes to biological activity; 3) to recognise inputs from water column organisms to the surface sediment from specific biological marker compounds; 4) to determine some of the diagenetic processes acting on lipids in the sediment by comparison of the lipid distributions in the surface sediment with those in a deeper sediment section; 5) to assess any changes in input between the time of deposition of the deeper section and the present; 6) to determine some of the factors affecting differences between free and bound lipid distributions and 7) to compare and contrast the results from Priest Pot with those from Coniston Water. The results of Chapter 3 are used to assign certain sedimentary lipids to a dinoflagellate origin.

Chapter 5 reports the free lipids of the surface and 15cm depth sediments of Lake Kinneret, Israel. The aims of this study were to 1) compare the sedimentary lipids with the lipids of the dominant primary producer in the lake, Peridinium cinctum (reported in Chapter 3); 2) to determine other sources of sedimentary lipids and 3) to study the early stages of lipid diagenesis in this sediment.

Chapter 6 gives the results of an investigation into the free lipids of Messel Shale, an ancient (Eocene) lacustrine sediment. The aims of this study were to 1) make use of advances in analytical chemical techniques to reappraise the lipid distributions in Messel Shale; 2) to extend the use of the biological marker compound information outlined in Chapters 2 to 5, to an ancient lacustrine sediment in order to assess the various inputs to this sediment and 3) to compare the lipid distributions present in a lacustrine sediment after 50×10^6 years with those present in supposedly analogous contemporary lacustrine sediments.

Chapter 7 describes the overall conclusions which may be drawn from this work, while Chapter 8 provides experimental details for all of the work described in this thesis.

CHAPTER TWO

CONISTON WATER

2.1 INTRODUCTION

2.1.i The lake

Coniston Water is situated in the English Lake District (Fig. 2.1/1) (Grid ref: SD 314 976), where it occupies a long narrow trough formed during the last glaciation (ca. 14000 years b.P.). The catchment (area 60.7 km^2) consists of Silurian slates and Borrowdale volcanic rocks separated by a narrow strip of Coniston Limestone. The lake (area 4.9 km^2; max. depth 56 m; mean depth 24 m) has a simple morphometry, consisting of one major basin, with a shallow basin at the northern end, situated above the major inflows. Coniston Water is classified as an oligo-mesotrophic lake, the contemporary trophic status (Jones et al., 1979) placing it approximately midway in the range observed for sixteen adjacent lakes (Pearsall, 1921). The oligo-mesotrophic status of the lake is reflected in a medium rate of sediment deposition (0.3 cm yr^{-1}) compared with thirteen other major lakes of the English Lake District (Pennington, 1981).

The distribution of alkyl esters, mid-chain ketones and fatty acids in the late-glacial and early post-glacial sediments of Coniston Water have been reported (Cranwell, 1984) and show that a qualitative record of autochthonous input is preserved in the

Fig. 2.1/1. A sketch map showing the location of Coniston Water, Cumbria.

sediment. These lipid distributions showed that there was a relatively greater autochthonous input during late-glacial and early post-glacial time, consistent with a higher trophic status caused by nutrient-rich conditions, and/or by more rapid colonisation by aquatic than by terrestrial biota (Cranwell, 1984).

The morphometry is such that, at the present trophic level, productivity never reaches such a rate as to cause the development of an anoxic water column. The bottom sediments are always oxic at the surface, with oxygen being depleted by ca. 2 cm sediment depth. The catchment consists mainly of pasture-land and woodland, with moorland on the upper slopes (Fig. 2.1/2), and is consistent with Coniston Water surficial sediment containing equivalent proportions of arboreal and grass pollen, amounting to 75% of total pollen (Professor W.Pennington, personal communication).

Under such conditions a relatively large higher plant contribution to the sediments, associated with terrestrial input, would be expected. The distribution of lipids of such allochthonous material is characteristic and well documented (Eglinton and Hunneman, 1967; Morrison and Bick, 1967; Cranwell, 1973). Generally, non-productive lakes such as Coniston Water, in which deoxygenation of the hypolimnion does not occur, have simpler sedimentary lipid assemblages than productive lakes in which autochthonous input is a greater proportion of the total and periodic anoxicity occurs. The reason is partly due to a more restricted species diversity and partly due to rapid removal of labile lipids under oxic conditions. Coniston Water provides

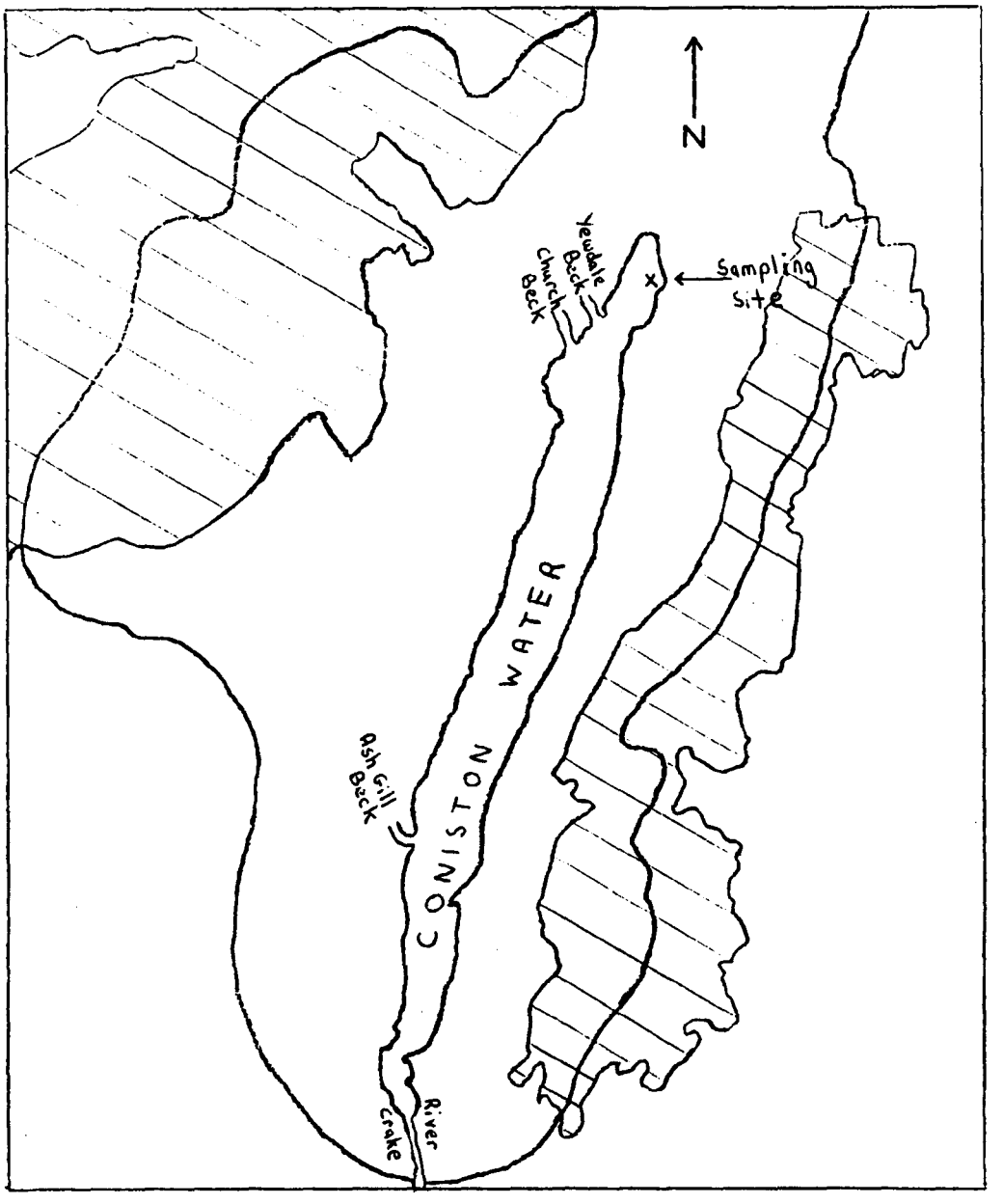


Fig. 2.1/2. Sketch map showing Coniston Water, with sampling site and boundary of catchment area marked. Shaded area is land above 230 m. Rough heathland and moorland occurs on the high ground. Most of the land on the east side of the lake is woodland, dominantly conifer plantations; some grassland occurs around the north and west of the lake.

a good contrast to Priest Pot, a nearby lake with high productivity and a seasonally anoxic hypolimnion (see Chapter 4). The sedimentary lipids should reflect the different inputs and post-depositional transformations, associated with different chemical environments and microbial organisms.

At the commencement of this work there was no published data on the organic geochemistry of Coniston Water. The sedimentary lipids were expected to be explicable in terms of input from terrestrially-derived higher plant material and a limited autochthonous production, with subsequent transformations mediated mainly by microbial organisms inhabiting the bottom sediments. Coniston Water was, therefore, chosen as a good example of a low productivity lake, in which to study the early stages of lipid diagenesis and to interpret the sedimentary lipid signature in terms of known sources. This chapter reports the distributions of free and bound hydrocarbons, fatty acids, ketones, alcohols and hydroxy acids of a short core of bottom sediment, of up to ca. 50 years age.

2.1.ii Elodea nuttallii

As part of the attempt to characterise the sedimentary lipid signature in terms of known sources, a sample of Elodea nuttallii (Planch.) St. John, a submerged macrophyte growing in the margins of Coniston Water, was collected for analysis of its lipids. The species is easily uprooted by wind induced turbulence. Such uprooted material was abundant at the sampling site (and is thus

a relevant possible source). The distribution of hydrocarbons, aldehydes, ketones, alcohols and fatty acids are reported and discussed in terms of a possible contribution to the sedimentary lipids.

2.2 RESULTS: CONISTON WATER

Tent Lodge bay, in the northern basin of Coniston Water was chosen as the sampling site for a number of reasons: it lies above the major inflows (Fig. 2.1/2) and thus was expected to have a more uniformly deposited sediment; survey cores had already shown that there was a considerable thickness of sediment and that the earliest sediments were accessible from this site; a full post-glacial core had been obtained and work on this was already in progress.

Two cores were taken from Tent Lodge bay (9/12/1984) at a water depth of 7 m, using a 1 m Mackereth corer (Mackereth, 1969). Cores of length 77 and 78 cm were obtained, having the following stratigraphic features:

0-2 cm mid-brown flocculent

2-19 cm black

19-24 cm grey-brown/black streaking, change in consistency to clay layer at 24 cm

24-43 cm grey-brown

>43 cm light brown

The bottom water pH was 6.2. In another core from the same site, a grey layer containing elevated levels of copper at 22-32 cm (Davison *et al.*, 1984) has been attributed to local copper mining which reached a peak in the 19 th century (Holland, 1981).

The cores were sectioned at 0-3 cm and 4-8 cm below the sediment-water interface and the corresponding fractions from the two cores combined. Analysis of the lipids from the 4-8 cm sections showed evidence for pollution of unknown origin, e.g. *n*-alkanes were bimodal with maxima at C_{17} and C_{25} and having a low CPI (1.2); GC revealed the presence of an envelope of unresolved components eluting between *ca.* *n*-C_{17} and *n*-C_{33} alkanes; pristane was present as two stereoisomers; a series of hopanes having the mature 17α(H),21β(H) stereochemistry, were present. In view of this, four new cores were collected north, south, east and west of the original cores but still within the 6.5 m water depth contour (21/4/1983), sectioned at 0-4 cm, 4-8 cm and 8-12 cm and their free hydrocarbons analysed by GC. The chromatograms obtained were all similar to that of the free hydrocarbons of the 0-3 cm section of the earlier cores, with no evidence of pollution. The 4-8 cm and 8-12 cm sections of the core taken from the east of the original cores, in which the stratigraphy was correlated with these previous cores, were used for the lipid analyses reported in this chapter.

Magnetic susceptibility data and comparison of stratigraphy with a dated core from the same site (Davison *et al.*, 1984) suggests the following dates for the three sections studied:

0-3 cm 1981-1973$\pm$2

4-8 cm 1970-1954±5

8-12 cm 1954±5-1932±10

The extracted lipids were separated chromatographically (Fig.2.2/1) and analysed by GC and GC-MS. Quantitation was determined by comparison of GC peak areas with those of standards.

Organic carbon, hydrogen and nitrogen are presented in Table 2.2/1. Lipid abundances by compound class are presented in Table 2.2/2.

Table 2.2/1 Organic carbon, hydrogen and nitrogen compositions* of Coniston Water sediments.

Section	% C	% H	% N	C:N
0-3 cm	9.18,9.73	1.72,1.76	0.54,0.62	17.0,15.7
4-8 cm	9.56,9.74	1.47,1.69	0.69,0.55	13.9,17.7
8-12 cm	9.97,9.68	1.68,1.62	0.54,0.63	18.5,15.4

* Values given are results of duplicate measurements.

2.2.i Hydrocarbons

a) n-Alkanes

Free n-alkanes maximised at C_{29} or C_{31} (Fig. 2.2/2). The relative proportion of bound $<C_{21}$ n-alkanes was greater than in the free hydrocarbons.

b) branched/cyclic hydrocarbons

Low levels of pristane, phytane and phytene were detected.

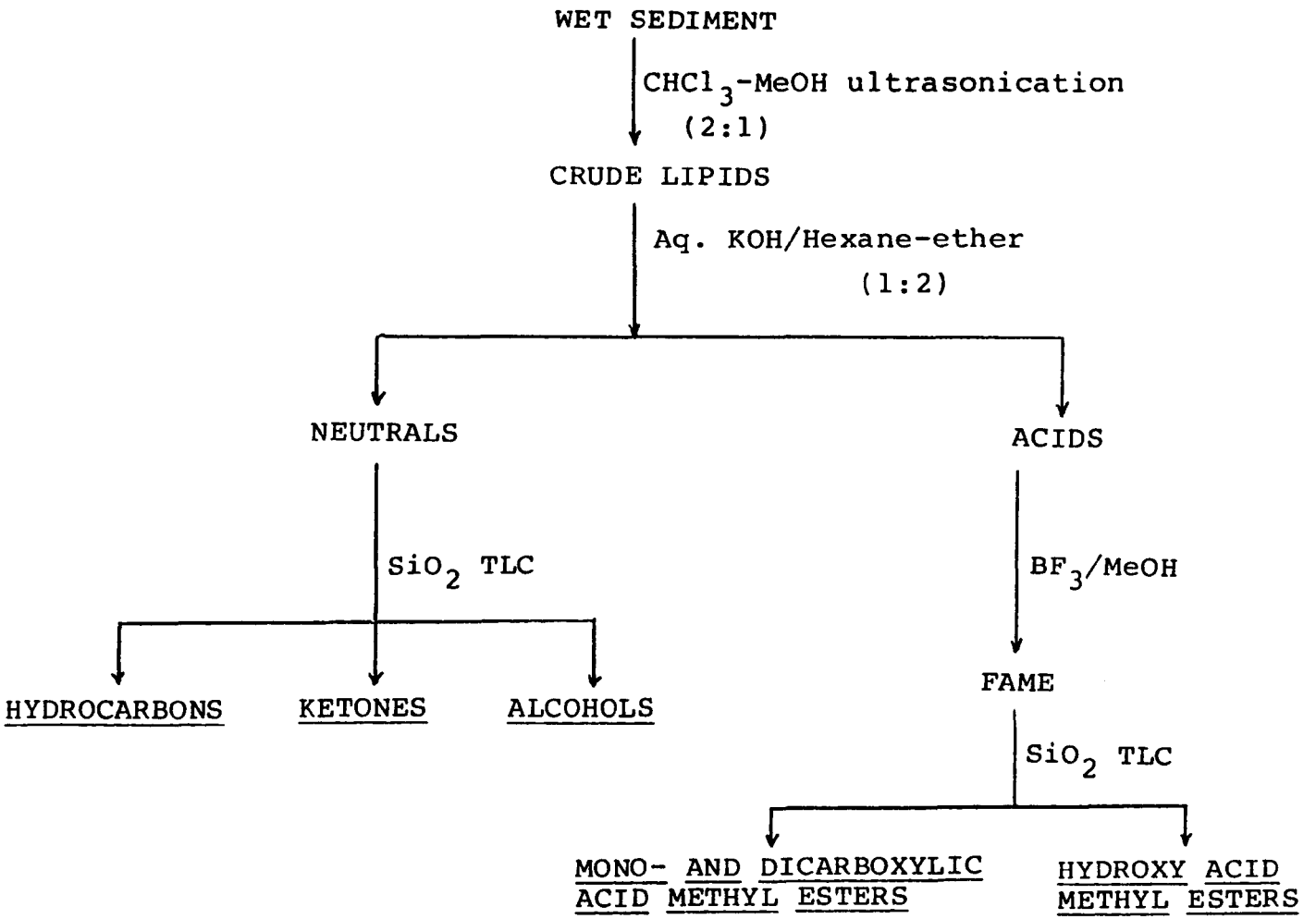


Fig. 2.2/1. Schematic diagram showing typical extraction and chromatographic separation of Coniston Water sediment lipids. Compound classes underlined refer to lipid content of various fractions as subsequently determined by GC-MS analysis. The extracted sediment residue was refluxed with 6N HCl (5 hours) and reextracted as above to yield bound lipids, which were chromatographically separated into compound classes as above.

Table 2.2/2 Compound class abundances[a] in Coniston Water sediment sections

Section	Hydrocarbons	Fatty acids[b]	Ketones	Alcohols	Sterols	Hydroxy acids
0 - 3 cm Free	22.4	104.8	2.9	36.2	129.9	12.2
Bound	2.3	1.2	0.8	2.2	1.1	10.8
4 - 8 cm Free	21.8	8.9	3.7	53.7	63.0	tr.
Bound	1.1	10.8	< 0.1	12.9	16.1	4.3
8 - 12 cm Free	13.7	6.3	1.8	18.6	28.3	tr.
Bound	3.0	43.1	< 0.1	1.6	< 0.1	10.7

(a) Amounts expressed in $\mu g\, g^{-1}$ dry, extracted sediment. Obtained by summing abundances of individual compounds. Quantitation determined by comparison of GC peaks with those of standards [$\underline{n}$-C_{18} and $\underline{n}$-C_{28} alkanes or $\underline{n}$-C_{18} alkanol (TMS) and cholesterol (TMS)].

(b) Includes mono- and dicarboxylic acids.

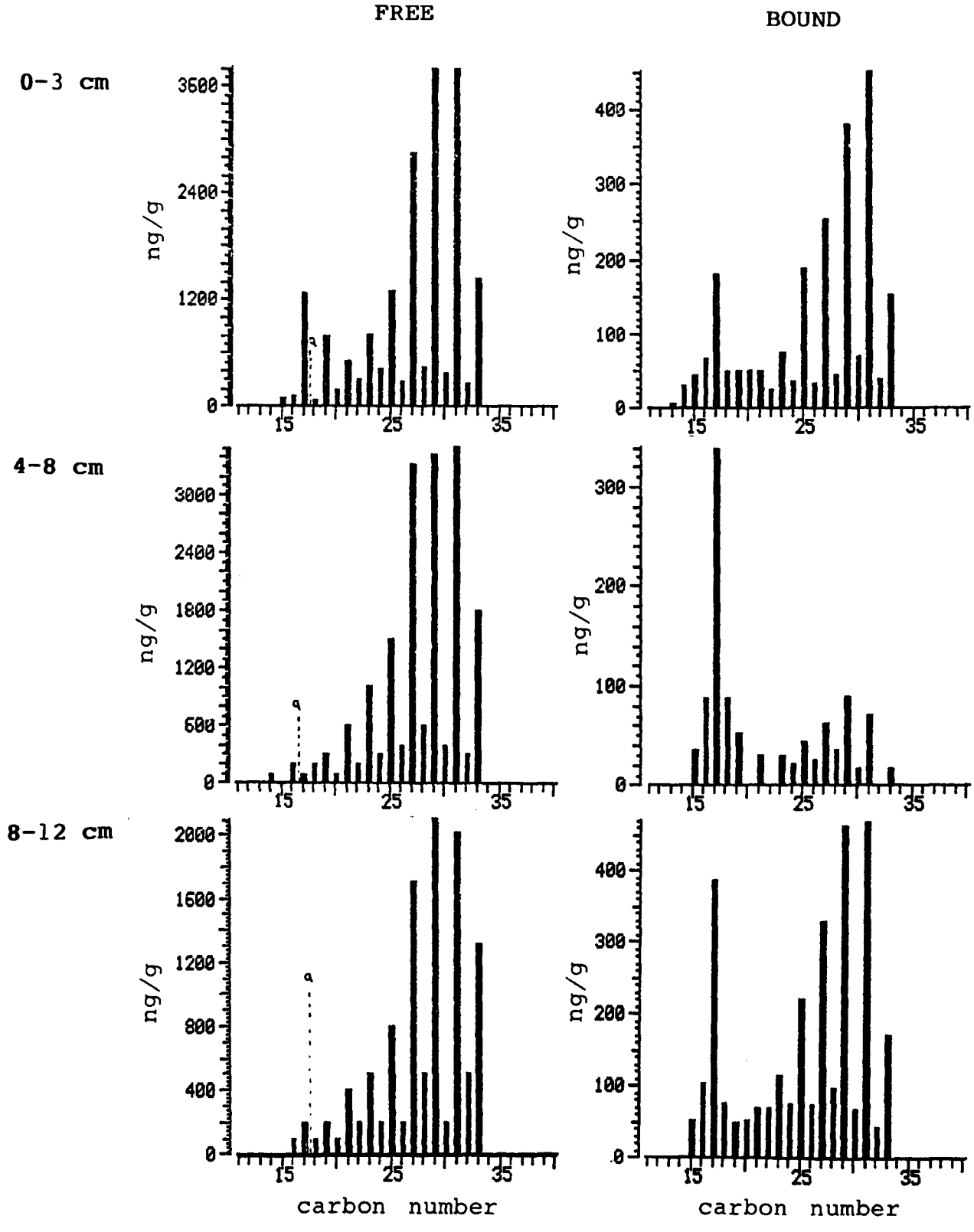


Fig. 2.2/2. Abundances of n-alkanes in Coniston Water sediment. Quantitation expressed as ng/g dry extracted sediment, determined by comparison of GC peak areas with those of known amounts of C_{18} and C_{28} n-alkanes.
(a) 2,6,10-Trimethyl-7(3-methylbutyl)-dodecane.

Gas chromatography and coinjection with standard pristane using a 60 m glass capillary column coated with DEGS/PEGS stationary phase, previously described by Maxwell *et al.* (1980), demonstrated that pristane was present as the 6R,10S stereoisomer (*meso*-form) in the free lipids of the 0-3 cm section. The free lipids also contained an additional acyclic isoprenoid alkane, eluting between n-C_{17} and pristane on OV1 liquid phase and identified as 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane (Yon *et al.*, 1982) the abundance of which increases with increasing depth, in contrast to the n-alkanes (Fig. 2.2/2). Trace levels of this compound were detected in the 0-3 cm bound lipids.

Hopanoid hydrocarbons were found in the free lipids, dominated by hop-22(29)-ene (0.5-1.5 μg g dry extracted sediment^{-1}) and also containing C_{29} 17α(H),21β(H)- and 17β(H),21β(H)-hopanes.

2.2.ii Fatty acids

a) Monocarboxylic acids

Straight chain monocarboxylic acids in the free lipid fractions decreased in abundance with increasing sediment depth, while those in the bound lipid fractions increased. Free acids showed a bimodal distribution, maximising at C_{16} and C_{24}, bound acids had essentially a unimodal distribution with C_{16} dominant (Fig. 2.2/3). The relative abundance of free C_{16} decreased with increasing depth.

Branched chain fatty acids were detected in all fractions

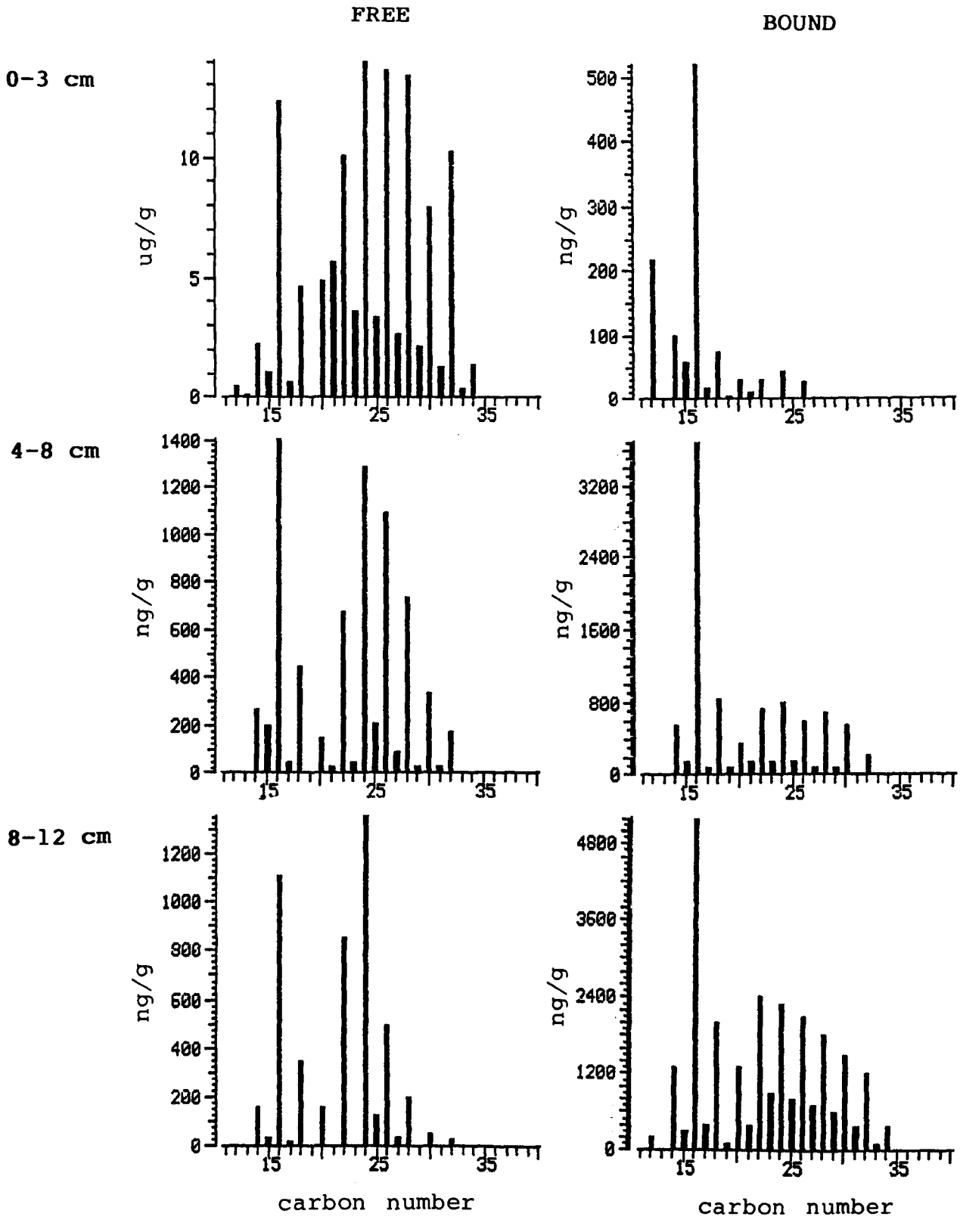


Fig. 2.2/3. Abundances of n-alkanoic acids in Coniston Water sediments. Quantitation expressed as ug/g (or ng/g) dry extracted sediment, determined by comparison of GC peak areas with those of known amounts of C_{18} and C_{28} n-alkanes.

(Table 2.2/3), but were relatively more abundant in the bound lipids than in the free. The dominant branched chain acids were iso- and anteiso-branched C_{15} compounds. One C_{32} cyclic monocarboxylic acid was detected, assigned as 17β(H),21β(H)-bishomohopanoic acid by comparison of its mass spectrum as a methyl ester with that of a standard, and was shown to be present as the 22R stereoisomer by co-chromatography with standard compounds (Fig. 2.2/4).

Three C_{16} and two C_{18} monoenoic acids were detected in the free lipids of the 0-3 cm section with a total abundance of 8.2 ug g^{-1}. Unsaturated acids were not detected in the free lipids of the deeper sections, but were present in low amounts (450 ng g^{-1} total abundance) in the bound lipids of the 4-8 cm section and in higher amounts (2.1 ug g^{-1} total abundance) in the bound lipids of the 8-12 cm section.

b) Dicarboxylic acids

A homologous series of α,w-dicarboxylic acids was present in each fraction. Their carbon number distributions are shown in Fig. 2.2/5. Free α,w-dicarboxylic acids maximised in the surficial 0-3 cm section, whereas the bound α,w-dicarboxylic acids were much more abundant in the 8-12 cm section than in the two shallower sections. α,w-Dicarboxylic acids present amongst the free lipids had a unimodal distribution, maximising at C_{22} or C_{20}, those present amongst the bound lipids had a bimodal distribution, with maxima at C_{16} and C_{22}.

The bound lipids of the 0-3 cm section contained two additional α,w-dicarboxylic acids which were not members of the homologous series. The mass spectra of these compounds are shown

Table 2.2/3 Branched chain and cyclic monocarboxylic fatty acids in Coniston Water sediment[a]

Compound	0 - 3 cm		4 - 8 cm		8 - 12 cm	
	Free	Bound	Free	Bound	Free	Bound
i C_{13}	250	-[b]	-	-	-	100
ai C_{13}	130	-	-	-	-	40
i C_{14}	500	20	-	70	-	300
i C_{15}	2400	180	100	240	50	900
ai C_{15}	3000	140	220	400	120	1200
i C_{16}	660	20	-	180	-	400
i C_{17}	660	< 10	30	140	-	800
ai C_{17}	530	10	30	210	-	500
2Me C_{17}	160	10	-	-	-	-
Phytanic	-	-	-	-	-	300
$C_{32}\beta\beta$[c]	2900	tr.	240	370	250	300

(a) Quantitation expressed as ng g dry extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of $\underline{n}$-C_{18} and $\underline{n}$-C_{28} alkanes.

(b) Not detected.

(c) 22R - 17β(H), 21β(H)-Bishomohopanoic acid.

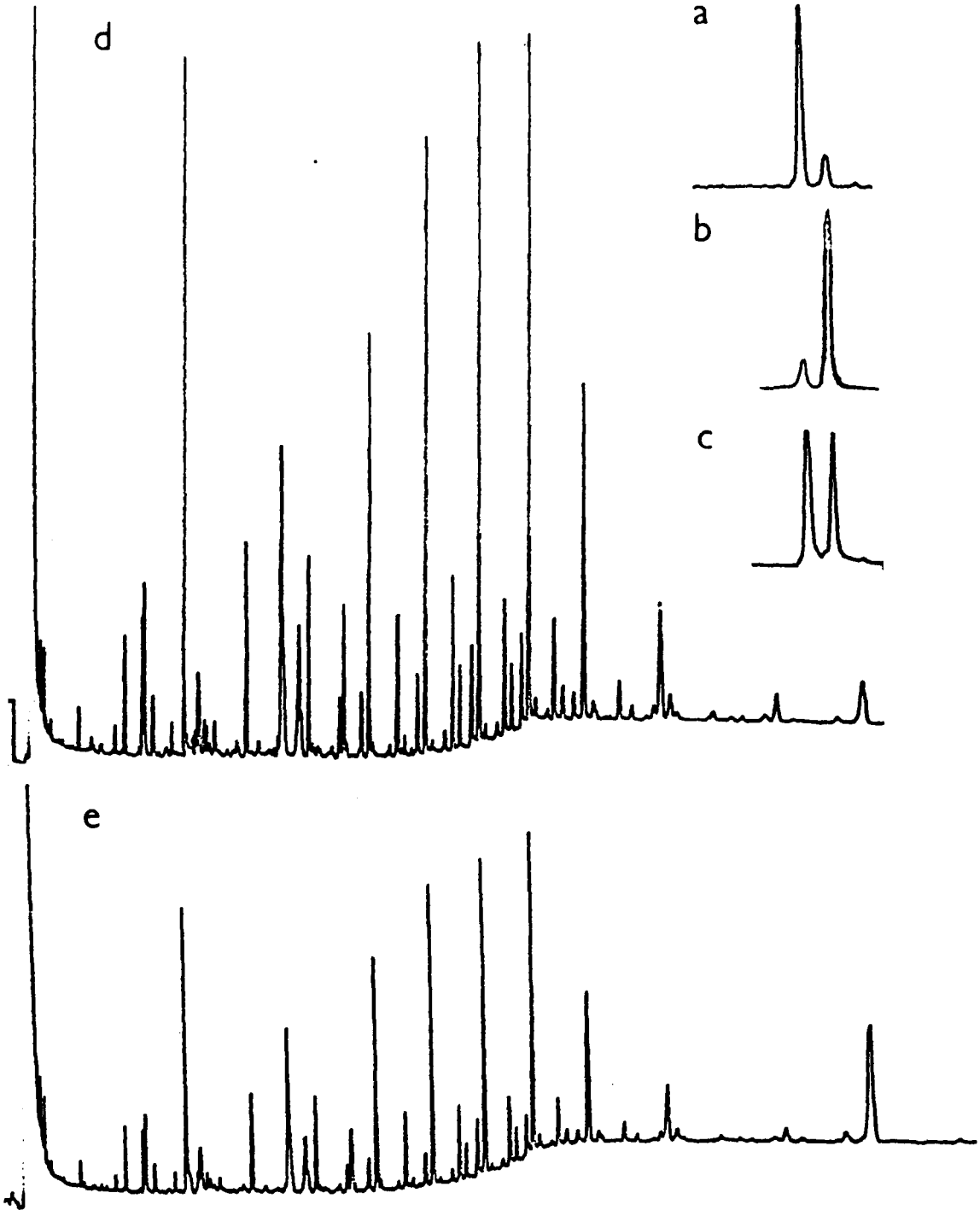


Fig. 2.2/4. Determination of stereochemistry at C-22 of 17β(H),21β(H)-bishomohopanoic acid in Coniston Water sediment.
a) Partial chromatogram of 22 S standard
b) Partial chromatogram of 22 R standard
c) Partial chromatogram of 22 S + 22 R standard
d) Chromatogram of Coniston Water 0-3 cm free acids
e) Chromatogram of Coniston Water 0-3 cm free acids + 22 R standard.
Conditions: 25 m x 0.3 mm CPSil 5 glass WCOT column (see Chapter 8); acids present as methyl ester derivatives.

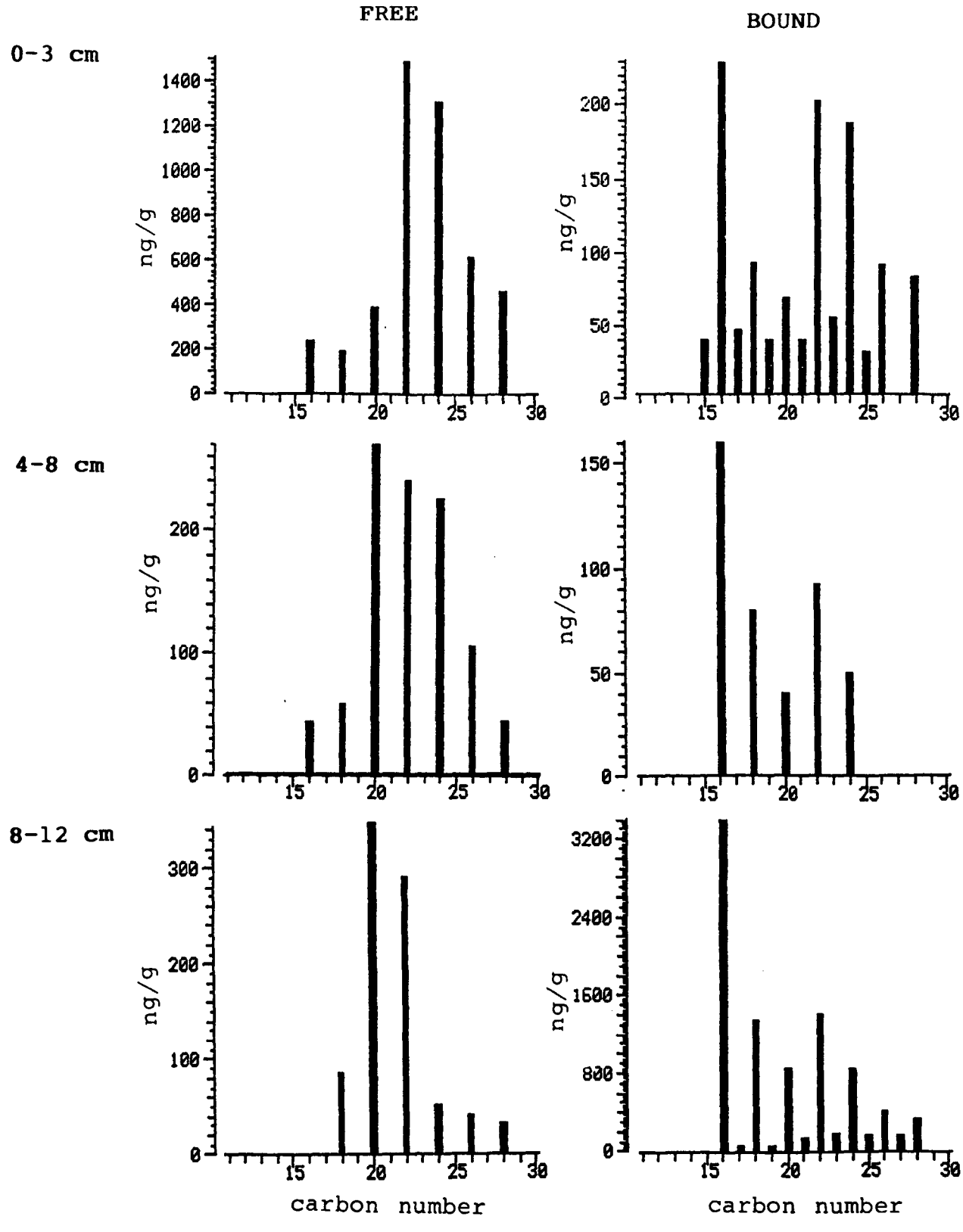


Fig. 2.2/5. Abundances of α,w-dicarboxylic acids in Coniston Water sediment. Quantitation expressed as ng/g dry extracted sediment, determined by comparison of GC peak areas with those of known amounts of C_{18} and C_{28} n-alkanes.

in Fig. 2.2/6. The mass spectrum of A is consistent with an α,w-dicarboxylic acid (as methyl ester) containing a methyl branch on one of carbons 2-6 (Ryhage and Stenhagen, 1965), the molecular ion at m/z 472 shows it to be a C_{28} compound having two degrees of unsaturation. Compound B has a molecular weight 14 a.m.u. higher and the occurrence of m/z 112 as base peak suggests that it contains a methyl branch on one of carbon atoms 2-6 at both ends of the chain. The size of the ions at m/z 87 and m/z 115 may indicate the branching to be on carbon atom 4:

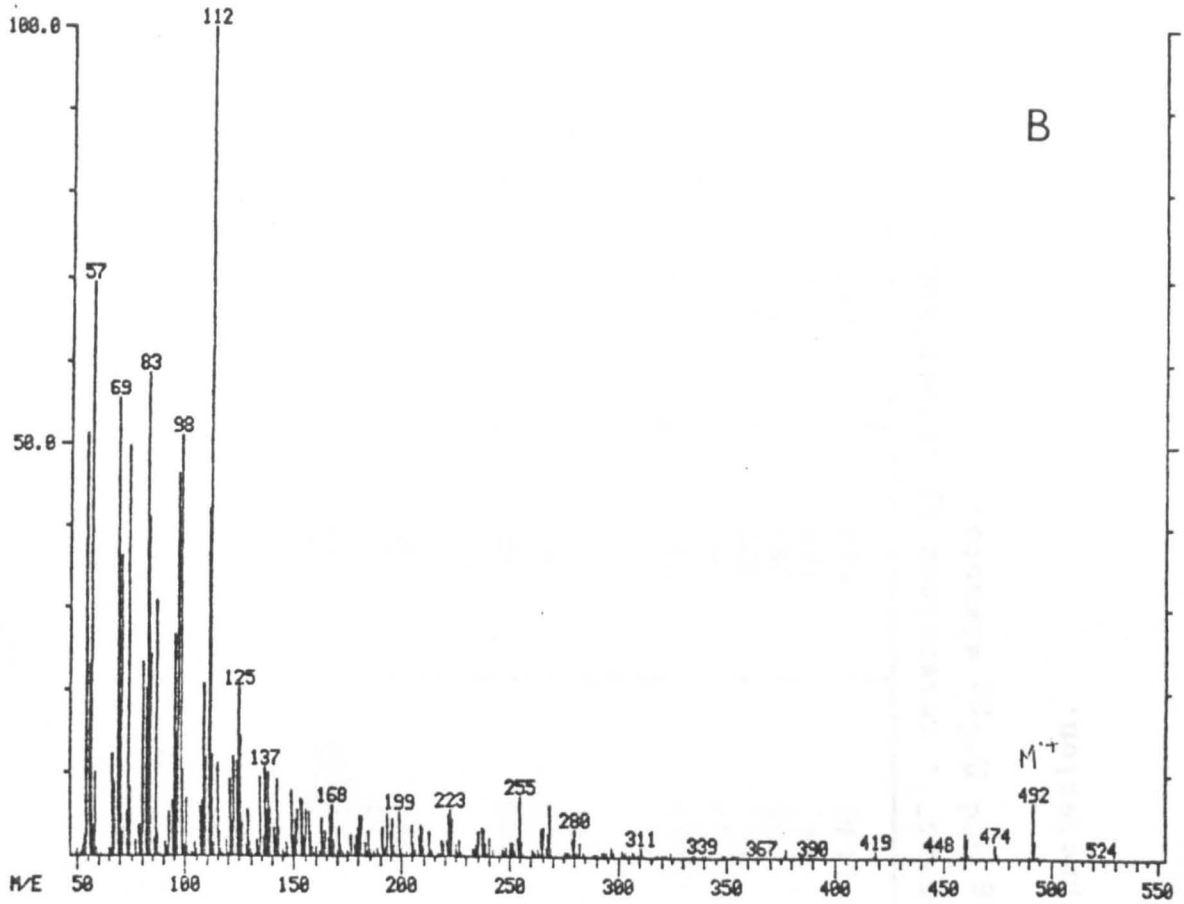
2.2.iii Ketones

a) Alkan-2-ones

6,10,14-Trimethylpentadecan-2-one was detected in each fraction. The free lipids contained a series of straight chain alkan-2-ones in the C_{17}-C_{34} range, having an odd over even carbon number predominance (CPI *ca.* 5) and maximising at C_{27}. These compounds reached a maximum total abundance of 1.4 ug/g in the 4-8 cm section.

b) Cyclic ketones

The free lipid fractions contained three series of cyclic ketones: steroidal-3-ones, hopanones and higher plant triterpenones. The abundances of individual compounds are presented in Table 2.2/4. Cholesta-3,5-dien-7-one and the 24-methyl and 24-ethyl pseudohomologues were detected in the bound lipid fractions (Table 2.2/5). Cyclic ketones present



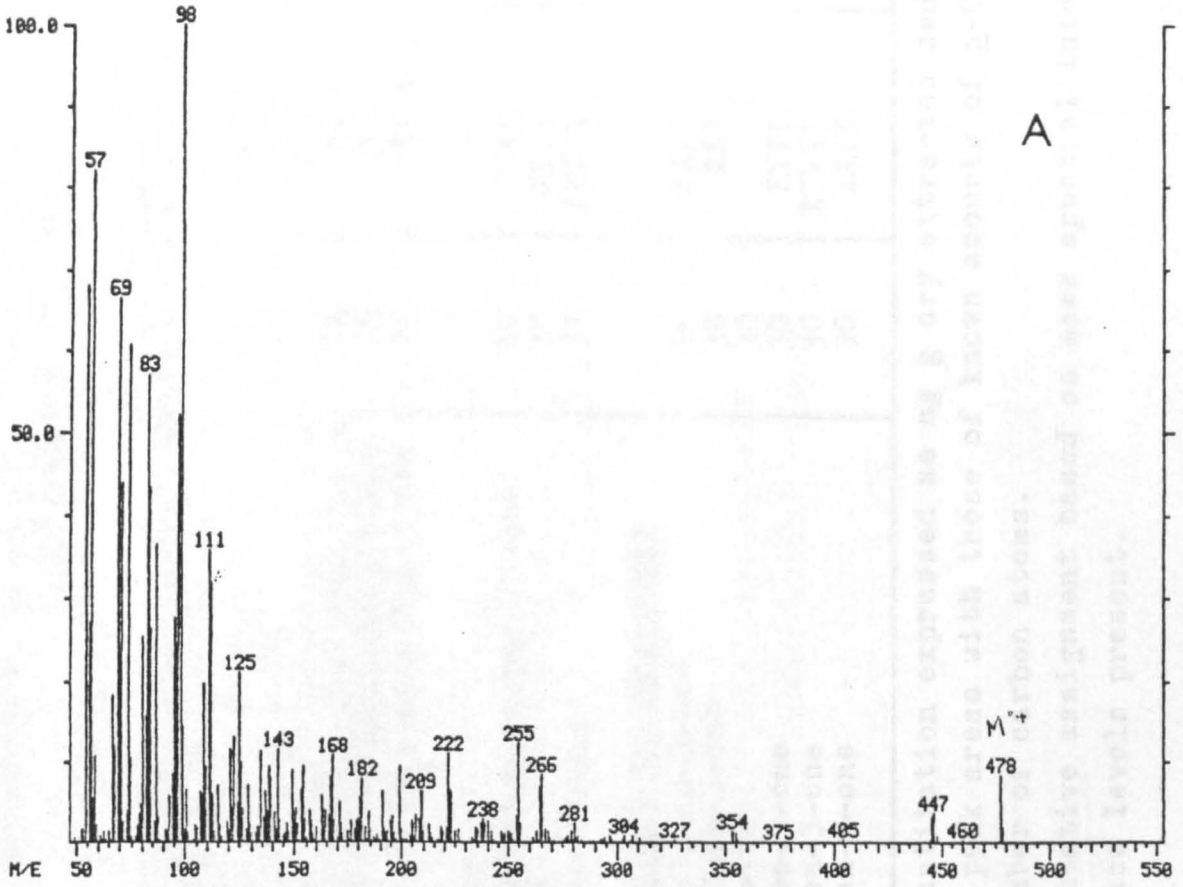


Fig. 2.2/6. Mass spectra of unknown α,w-dicarboxylic acids (as methyl esters) isolated from Coniston Water 0-3 cm bound lipids, see text for details. Instrumental conditions are given in Chapter 8.

Table 2.2/4 Abundances[a] of cyclic ketones in the free lipid fractions of Coniston Water sediment

Compound	C no[b]	Structure	0 - 3 cm (ng g^{-1})	4 - 8 cm (ng g^{-1})	8 - 12 cm (ng g^{-1})
Steroidal ketones					
5β(H)-Cholestan-3-one	27	XIV a	150	10	3
5α(H)-Cholestan-3-one	27	XV a	60	40	13
24-Methyl-5β(H)-cholestan-3-one	28	XIV e	70	10	3
24-Methyl-5α(H)-cholestan-3-one	28	XV e	tr.[d]	20	6
24-Ethyl-5β(H)-cholestan-3-one	29	XIV h	260	40	14
24-Ethyl-5α(H)-cholestan-3-one	29	XV h	350	190	60
Hopanones					
22,29,30-Trisnorhopan-21-one	27	XL	190	90	29
Hopanone	30	XLII	100	20	5
Homohopan-29-one	31	XLIII	tr.	30	10
Higher plant triterpenones					
Taraxer-14-en-3-one	30	XXIV	60	270	180
Olean-12-en-3-one	30	XXV	40	160	110
Lupenone[c]	30		tr.	300	200
Urs-12-en-3-one	30	XXVI	30	210	140
Glut-5-en-3-one	30	XXVII	tr.	350	230
Friedelan-3-one	30	XXIX	1130	640	420

(a) Quantitation expressed as ng g dry extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of n-C_{18} and n-C_{28} alkanes.

(b) Number of carbon atoms.

(c) Tentative assignment based on mass spectral interpretation.

(d) Trace levels present.

Table 2.2/5 Abundances[(a)] of cyclic ketones in the bound lipid fractions of Coniston Water sediment

Compound	C no.[(b)]	Structure	0 - 3 cm (ng g^{-1})	4 - 8 cm (ng g^{-1})	8 - 12 cm (ng g^{-1})
Cholesta-3,5-dien-7-one	27	XVIII a	140	15	200
24-Methylcholesta-3,5-dien-7-one	28	XVIII e	70	5	70
24-Ethylcholesta-3,5-dien-7-one	29	XVIII h	540	40	440

(a) Quantitation expressed as ng g dry extracted $sediment^{-1}$, determined by comparison of GC peak areas with those of known amounts of $\underline{n}$-C_{18} and $\underline{n}$-C_{28} alkanes.

(b) Number of carbon atoms.

amongst the bound lipids were absent from the free lipids and *vice versa*.

2.2.iv Alcohols

a) Alkanols

Free *n*-alkanols increased in abundance between the 0-3 cm and 4-8 cm sections and then decreased in abundance in the 8-12 cm section, the maximum at C_{26} in the 0-3 cm and 4-8 cm sections being replaced by a maximum at C_{22} in the 8-12 cm section (Fig. 2.2/7). Bound *n*-alkanols were much more abundant in the 4-8 cm section than in the other two sections and contained a very large C_{22} component (Fig. 2.2/7). The proportion of $< C_{22}$ alkanols was higher amongst the bound lipids than the free lipids. *Iso*- and *anteiso*-branched chain alkanols ranging from C_{13} to C_{17}, dominated by *iso*- and *anteiso*-C_{15}, were present in higher relative abundances amongst the bound lipids than the free. Phytol present amongst the free lipids decreased in abundance with depth from 10.4 ug g^{-1} in the 0-3 cm section to 4.4 ug g^{-1} in the 8-12 cm section. The bound lipids contained low amounts of phytol (< 180 ng g^{-1}).

Two C_{16} monounsaturated alkanols were detected amongst the free lipids of the 0-3 cm section (900 and 220 ng g^{-1} respectively). Unsaturated alkanols, other than phytol, were negligible in all other fractions.

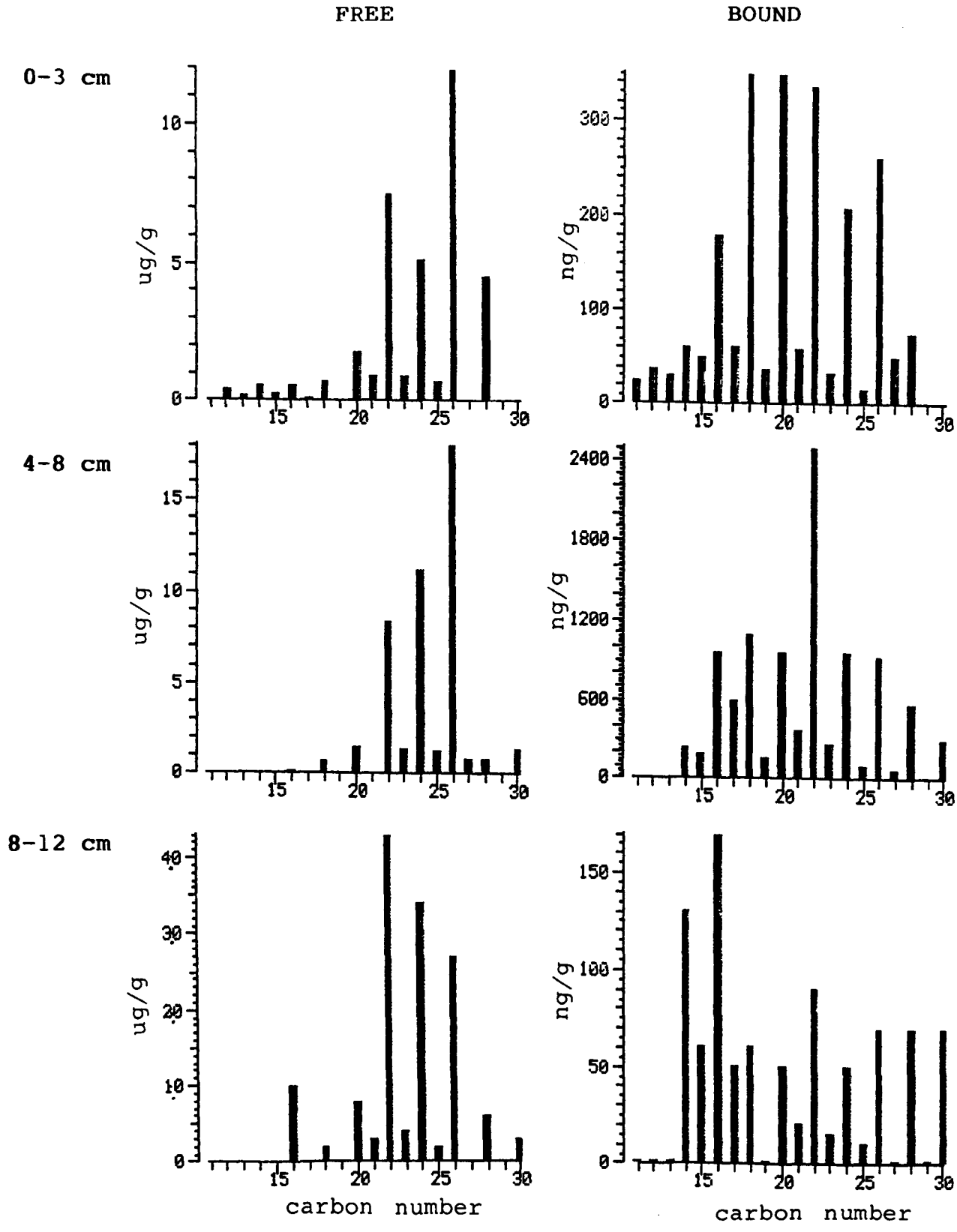


Fig. 2.2/7. Abundances of n-alkanols in Coniston Water sediments. Quantitation expressed as ug/g (or ng/g) dry extracted sediment, determined by comparison of GC peak areas with that of a known amount of C_{18} n-alkanol, all present as TMS ethers.

b) Cyclic alcohols

The major components of the cyclic alcohols were sterols, with lower amounts of hopanoid and higher plant triterpenoid alcohols also being detected. Sterol abundances are given in Table 2.2/6 and their distributions are shown diagrammatically in Fig. 2.2/8. The sterols were dominated by 24-ethyl cholest-5-en-3β-ol. Generally a lower Δ^5:5α(H) ratio with increasing depth was observed for this compound (6.5→ 2.1→ 2.5) and the C_{27} (2.6 → 0.75 → 0.7) and C_{28} (∞ → 0.7 → 0.5) pseudohomologues. Free sterols decreased in abundance with increasing sediment depth. Bound sterols were markedly more abundant in the 4-8 cm section than in the 0-3 cm or 8-12 cm sections. Unlike the free sterols, bound sterols did not show a marked increase in Δ^5:5α(H) ratio with increasing depth; also, the relative importance of the C_{27} components was greater amongst the bound lipids of the 4-8 cm and 8-12 cm sections than for the free lipids.

The abundances of hopanoid and higher plant triterpenoid alcohols present in the free lipid fractions are given in Table 2.2/7; they were absent from the bound lipids (or only present in trace levels).

2.2.v Hydroxy acids

2- And 3-hydroxy acids of the same carbon number and skeleton coeluted on OV1 coated GC columns when analysed as methyl ester,

Table 2.2/6 Abundances of sterols in Coniston Water sediment

No.[a]	Compound	C no.[b]	Structure	0 - 3 cm		4 - 8 cm		8 - 12 cm	
				(c) Free	(d) Bound	(c) Free	(c) Bound	(c) Free	(d)(e) Bound
1.	5β(H)-cholestan-3β-ol	27	II a	1.1	10	tr(f)	0.1	tr.	-(g)
2.	5β(H)-cholestan-3α-ol	27	V a	0.9	-	tr.	-	tr.	-
3.	Cholesta-5,22-dien-3β-ol	27	VI c	1.8	-	1.1	0.6	0.4	-
4.	5α(H)-cholest-22-en-3β-ol	27	III c	1.2	-	-	-	-	-
5.	Cholest-5-en-3β-ol	27	VI a	11.1	110	4.2	2.9	1.4	230
6.	5α(H)-Cholestan-3β-ol	27	III a	4.3	30	5.6	0.6	2.0	40
7.	24-Methylcholesta-5,22-dien-3β-ol	28	VI f	10.2	80	2.3	0.1	0.6	-
8.	24-Methyl-5α(H)-cholest-22-en-3β-ol	28	III f	2.6	-	-	-	-	-
9.	4α-Methyl-5α(H)-cholest-8(14)-en-3β-ol	28	XI a	2.3	-	1.8	-	tr.	-
10.	24-Methylcholest-5-en-3β-ol	28	VI e	8.2	80	1.6	0.5	0.6	tr.
11.	24-Methyl-5α(H)-cholestan-3β-ol	28	III e	tr.	30	2.3	0.1	1.3	tr.
12.	24-Ethyl-5β(H)-cholestan-3β-ol	29	II h	3.2	-	3.2	0.4	1.3	-
13.	24-Ethyl-5β(H)-cholestan-3α-ol	29	V h	2.8	-	0.4	0.2	0.1	-
14.	24-Ethylcholesta-5,22-dien-3β-ol	29	VI g	9.8	110	4.9	1.0	2.3	-
15.	24-Ethyl-5α(H)-cholest-22-en-3β-ol	29	III g	1.2	-	-	-	-	-
16.	24-Ethylcholest-5-en-3β-ol	29	VI h	61.1	610	24.0	7.5	12.7	80
17.	24-Ethyl-5α(H)-cholestan-3β-ol	29	III h	9.3	80	11.4	1.9	5.5	70

(a) Refers to histogram bar number in Fig. 4.2/8.

(b) Number of carbon atoms.

(c) Quantitation expressed as μg g dry extracted sediment^{-1}, determined by comparison of GC peak areas with that of a known amount of cholesterol, all derivatised as TMS ethers.

(d) Quantitation expressed as ng g dry extracted sediment^{-1}, determined as above.

(e) Low amounts made quantitation unreliable.

(f) Trace amount present.

(g) Not detected.

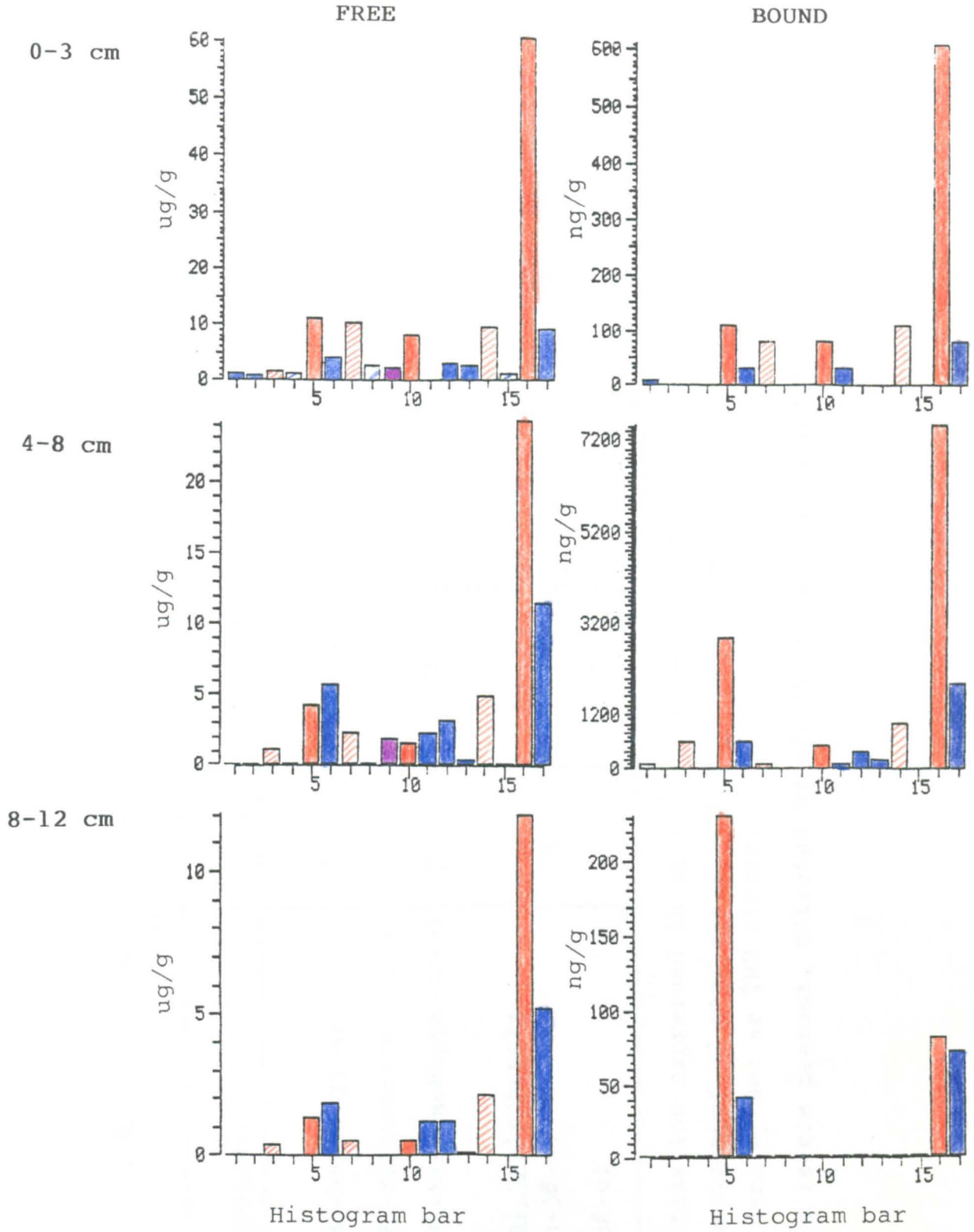


Fig. 2.2/8. Distribution of sterols in Coniston Water sediment. Compound identifications and abundances are given in Table 2.2/5. Histogram bar number refers to number in first column of Table 2.2/5.

Δ^0 - Δ^5 - 4Me -

Hatching indicates presence of Δ^{22}

Table 2.2/7 Abundances[a] of free triterpenoid alcohols in Coniston Water sediment

Compound	C no.	Structure	0 - 3 cm ($\mu g\ g^{-1}$)	4 - 8 cm ($\mu g\ g^{-1}$)	8 -12 cm ($\mu g\ g^{-1}$)
Hopanols					
17β(H)21β(H)-hopan-29-ol	30	XXXVII	tr.[b]	1.0	0.6
17α(H)21β(H)-bishomohopan-32-ol	32	XXXVIII	tr.[b]	0.5	0.4
17β(H)21β(H)-bishomohopan-32-ol	30	XXXIX	tr.[b]	1.4	0.8
Higher plant triterpenols					
Olean-12-en-3β-ol	30	XXI	2.8	1.4	0.9
Urs-12-en-3β-ol	30	XXII	3.7	6.3	1.3

(a) Quantitation expressed in $\mu g\ g^{-1}$ of dry extracted sediment, determined by comparison of GC peak areas with that of a known amount of n-C_{28} alkanol, all derivatised as TMS ethers.

(b) Trace levels present, detected by m/z 191 mass fragmentogram.

TMS ethers. The resultant mixed mass spectra could, however, be interpreted to estimate their relative proportions by comparison of characteristic fragment ions (Eglinton et al., 1968).

Low levels of 2-hydroxy acids were detected amongst the free lipids of the 0-3 cm section, ranging from C_{16} to C_{28} and maximising at n-C_{24} (900 ng g^{-1}), with some iso- and anteiso-branched C_{15} compounds also present. 2-Hydroxy acids were not detected amongst the free lipids of the two deeper sections. Similarly, bound 2-hydroxy acids were only of detectable levels in the 0-3 cm section, where they were present in trace amounts at n-C_{22}, n-C_{23} and n-C_{24}.

The free lipids of the 0-3 cm section contained very low levels of 3-hydroxy acids in the C_{10}-C_{28} range including iso- and anteiso-branched C_{15} and C_{17} compounds. Free 3-hydroxy acids were of negligible concentration at the two deeper depths. Bound 3-hydroxy acids were detected ranging from C_7 to C_{18}, with a high predominance of even carbon number straight chain components, odd carbon number compounds being dominated by branched, especially iso- and anteiso-, components (Fig. 2.2/9). A minimum abundance of bound 3-hydroxy acids in the 4-8 cm sediment section was observed. The proportion of shorter chain compounds diminished with increasing sediment depth.

w-Hydroxy acids (C_{12}-C_{28}) were detected with a bimodal distribution, maxima occurring at C_{16} and C_{22} (Fig. 2.2/10). The bound w-hydroxy acids had a relatively larger C_{16} homologue than those present in a free form. Both free and bound w-hydroxy acids showed a decrease in abundance between the 0-3 cm and 4-8 cm sections, increasing thereafter in the 8-12 cm section.

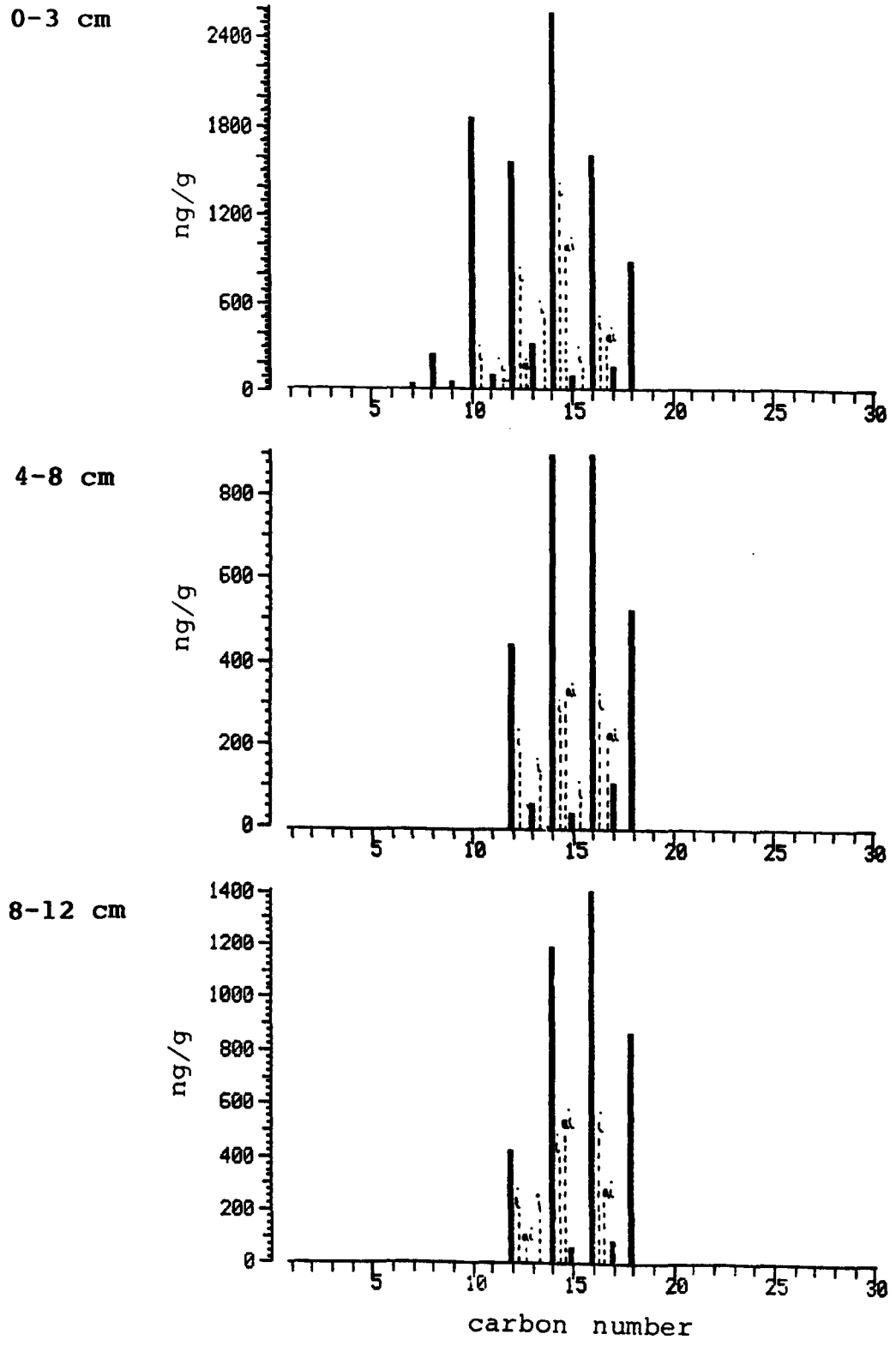


Fig. 2.2/9. Abundances of bound 3-hydroxy acids in Coniston Water sediments. Quantitation expressed as ng/g dry extracted sediment, determined by comparison of GC peak areas with those of known amounts of C_{18} and C_{28} n-alkanes. i = iso-branched; ai = anteiso-branched.

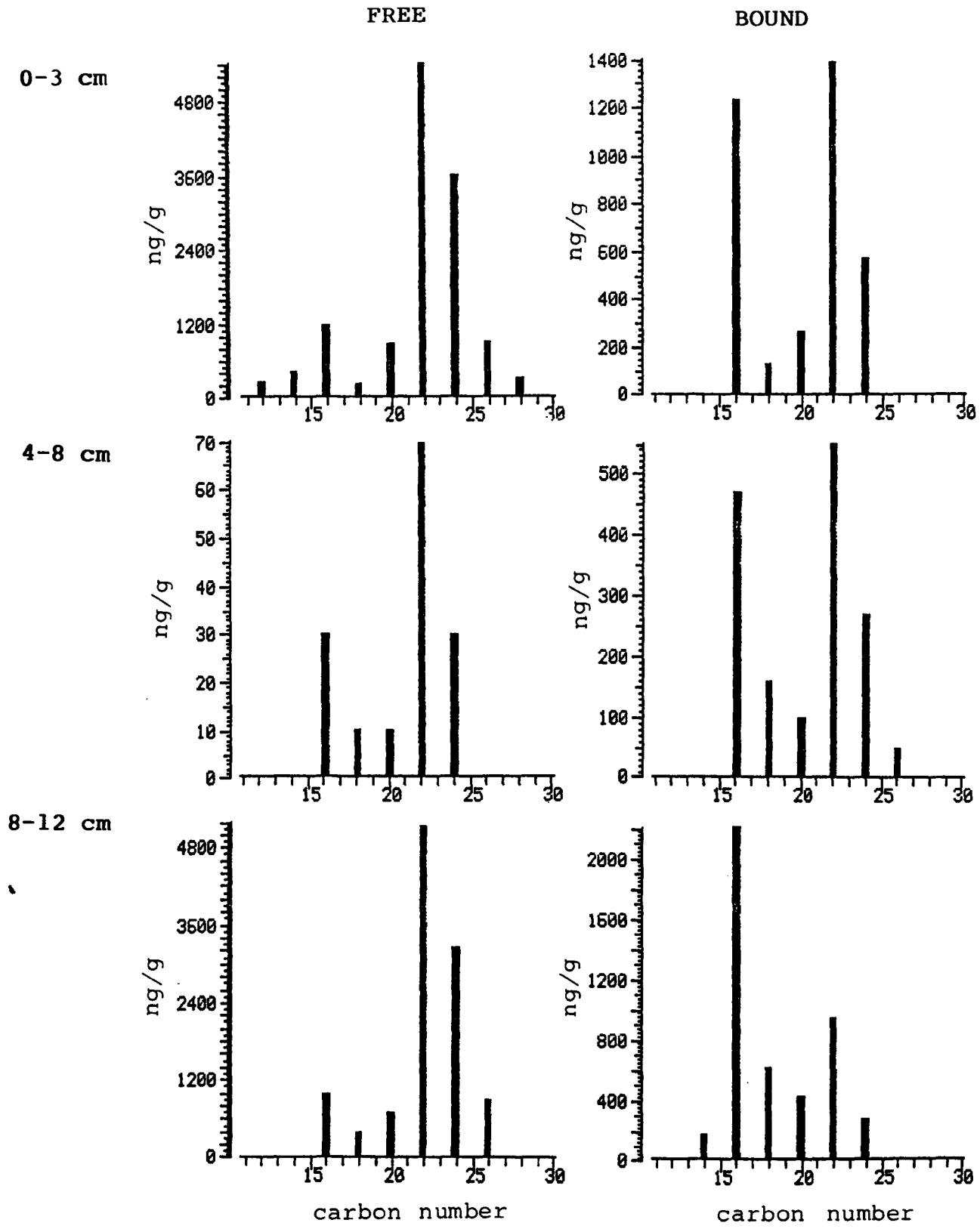


Fig. 2.2/10. Abundances of w-hydroxy acids in Coniston Water sediments. Quantitation expressed as ng/g dry extracted sediment, determined by comparison of GC peak areas with those of known amounts of C_{18} and C_{28} n-alkanes.

2.3 RESULTS: ELODEA nuttallii

A sample of Elodea nuttallii growing in the margins of Coniston Water, was collected during February 1983 at a time when epiphytes should have been at a minimum, but when growth was occurring in the plant. The absence of epiphytes was subsequently confirmed by microscopic investigation. Maceration with scissors, followed by extraction with organic solvents and chromatographic separation yielded lipid fractions containing hydrocarbons, aldehydes, ketones, alcohols and fatty acids.

2.3.i Hydrocarbons

n-Alkanes were detected between C_{17} and C_{23} with traces of n-C_{24}-C_{28} (Fig.2.3/1). A small amount of a phytadiene was also present.

2.3.ii Aldehydes

Saturated aldehydes were detected at C_{14}, C_{15} and C_{17}. Monounsaturated aldehydes were detected at C_{15} and C_{17} and additionally a C_{17} triunsaturated aldehyde was present.

2.3 RESULTS: ELODEA nuttallii

A sample of Elodea nuttallii growing in the margins of Coniston Water, was collected during February 1983 at a time when epiphytes should have been at a minimum, but when growth was occurring in the plant. The absence of epiphytes was subsequently confirmed by microscopic investigation. Maceration with scissors, followed by extraction with organic solvents and chromatographic separation yielded lipid fractions containing hydrocarbons, aldehydes, ketones, alcohols and fatty acids.

2.3.i Hydrocarbons

n-Alkanes were detected between C_{17} and C_{23} with traces of n-C_{24}-C_{28} (Fig.2.3/1). A small amount of a phytadiene was also present.

2.3.ii Aldehydes

Saturated aldehydes were detected at C_{14}, C_{15} and C_{17}. Monounsaturated aldehydes were detected at C_{15} and C_{17} and additionally a C_{17} triunsaturated aldehyde was present.

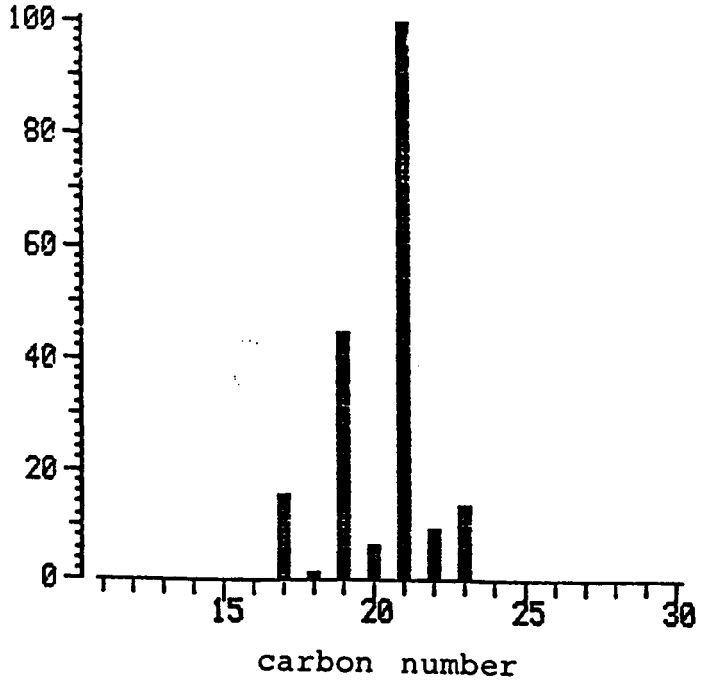


Fig. 2.3/1. Distribution of n-alkanes in Elodea nuttallii collected from Coniston Water.

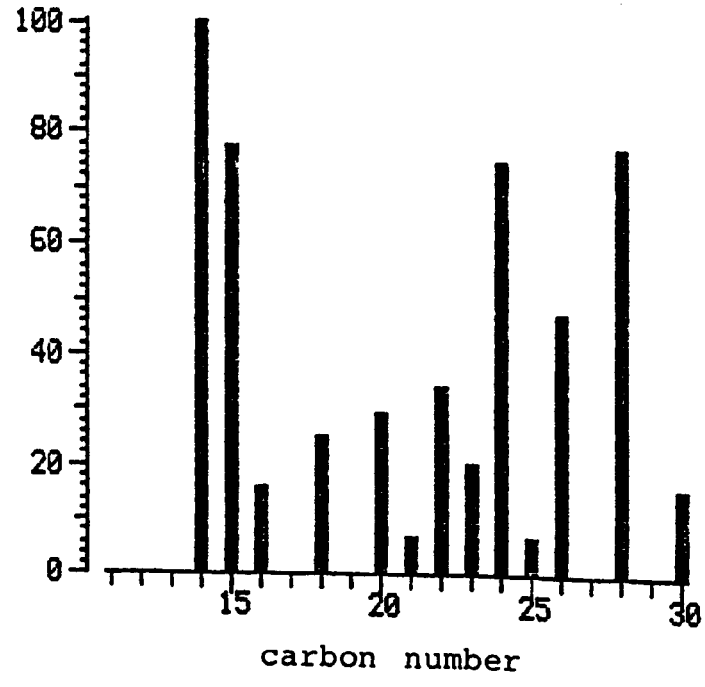


Fig. 2.3/2. Distribution of n-alkanols in Elodea nuttallii collected from Coniston Water.

2.3.iii Ketones

A small amount of 6,10,14-trimethylpentadecan-2-one was detected and, at an order of magnitude more abundant, 24-ethylcholest-4-en-3-one.

2.3.iv Alcohols

Alcohols were the most abundant lipid fraction in the extract of *Elodea nuttallii* *n*-Alkanols were present from C_{14}-C_{30}, maximising at C_{14} and C_{28} (Fig. 2.3/2). Phytol was very abundant. The sterols had a simple distribution, consisting of cholest-5-en-3β-ol, 24-methylcholesta-5,22-dien-3β-ol, 24-methylcholest-5-en-3β-ol, 24-ethylcholesta-5,22-dien-3β-ol and 24-ethylcholest-5-en-3β-ol, with the last dominant.

2.3.v Fatty acids

Monocarboxylic acids were found in low abundance; possibly the majority of the fatty acid content was present in an esterified form as triglycerides, which were not analysed. The major *n*-alkanoic acid was C_{16}, with C_{18} an important component. Unsaturated acids were dominated by C_{18} and C_{16} species, a GC peak containing $C_{18:2}$ and $C_{18:3}$ acids (as methyl esters) having an area 70% that of the *n*-C_{16} alkanoic acid.

2.4 DISCUSSION

2.4.i Sources of lipids in Coniston Water sediment

Sedimentary C:N ratios have been used to estimate the relative importance of different inputs of organic matter (Ishiwatari *et al.*, 1980; Meyers *et al.*, 1984). The high C:N ratios observed in the three sediment sections of Coniston Water suggest that allochthonous material was an important source of organic matter to these sediments. *n*-Alkanes, *n*-alkanols and *n*-alkanoic acids having a chain length $>C_{22}$ are indicative of a higher plant input (Eglinton and Hunneman, 1967; Cranwell, 1973, 1974; Brooks *et al.*, 1976; Cardoso *et al.*, 1983). The abundance of such compounds in the sediments of Coniston Water suggests that terrestrial higher plants are the major source of organic matter in the sediments. The predominance of 24-ethylcholest-5-en-3β-ol is consistent with a major allochthonous input (e.g. Huang and Meinschein, 1979), as are the large amounts of higher plant triterpenoid ketones present amongst the free lipid fractions (Table 2.2/4).

Although the major sources of organic matter in the Coniston Water sediment samples was allochthonous in nature, an autochthonous input could still be recognised from the composition of sedimentary lipids. Thus the maximum at C_{16} in the bimodal distribution of free *n*-alkanoic acids reflects a

microbial input. Iso- and anteiso-branched acids and alcohols, thought to originate from bacteria (Parker, 1969; Cranwell, 1980), were recognised in Coniston Water sediments. Similarly the presence of bound 3-hydroxy acids ranging from C_{10}-C_{18} with odd carbon number components dominated by iso- and anteiso-branched species, has been taken as reflecting a microbial input, originating from bacterial cell walls (Cardoso et al., 1977; Boon et al., 1977; Cranwell, 1981b; Cardoso and Eglinton, 1983; Klok, 1984). The small maximum at C_{17} in the distribution of the free n-alkanes of the 0-3 cm section reflects an input from aquatic microorganisms (e.g. Giger et al., 1980). Coniston Water sediments, however, contain relatively less lipids of autochthonous origin than in a productive lake such as Rostherne Mere (Gaskell and Eglinton, 1976; Brooks et al., 1976; Cardoso et al., 1983).

The pristane in the Coniston Water sediment sections analysed, was shown to be present as a single stereoisomer having the 6R,10S stereochemistry. Thus it does not arise from pollution of the sediment, but is consistent with a direct biological origin from phytol, possibly arising via degradation of chlorophyll in the gut of zooplankton (Brooks et al., 1978). 2,6,10-Trimethyl-7-(3-methylbutyl)-dodecane (XLVII) does not have a well characterised origin. It has been suggested that it is specific to the marine environment (Barrick et al., 1980), but this is clearly not the case as it is present in Coniston Water sediment and has been reported to occur in other freshwater sediments (Cranwell, 1978; Yon et al., 1982). Hop-22(29)-ene, the dominant hopanoid hydrocarbon observed in Coniston Water

sediments, has been reported to occur in bacteria (Ourisson et al., 1979).

w-Hydroxy acids are present in both microorganisms and higher plants, where they may exist in a free form in cuticular waxes or in a bound form in the polymers cutin (C_{16}, C_{18}) and suberin (C_{16}-C_{24}) (e.g. Holloway, 1973). They may also be formed by microbial transformation of other lipid classes, such as alkanoic acids (Boon et al., 1977). Similar distributions of free and bound w-hydroxy acids to those found in Coniston Water sediment have been reported for surface sediments of Rostherne Mere, a productive lake, and for a 5000 year old lacustrine sediment from Esthwaite Water (Cardoso et al., 1977; Cardoso and Eglinton, 1983).

The distributions of free α,w-dicarboxylic acids resemble those of the free w-hydroxy acids (cf. Figs. 2.2/5 and 2.2/10), but there is not a close enough match to suggest that the former arises from the latter by microbial oxidation, as has been proposed for some sediments (Cranwell, 1977). Also the increase in abundance of free α,w-hydroxy acids between the 4-8 cm and 8-12 cm sections is not paralleled by an increase in abundance of free α,w-dicarboxylic acids. This does not rule out a partial origin of α,w-dicarboxylic acids from microbial oxidation of w-hydroxy acids, which, for compounds derived from terrestrial detritus, may proceed before deposition in the lake. Thereafter, differences in diagenesis and new inputs may alter the distributions so that they do not match.

The bound α,w-hydroxy acids also have distributions closely resembling those of the bound w-hydroxy acids (Figs. 2.2/5 and

2.2/10), although w-hydroxy acids were not detected above C_{26}. In the case of the bound lipids an increase in abundance of w-hydroxy acids between the 4-8 cm and 8-12 cm sections and an increased proportion of homologues below C_{20} is paralleled by an increase in abundance and similar change in distribution of α,w-dicarboxylic acids, indicating that the two classes of compounds share a common origin. Similar changes in abundance and distribution of bound w-hydroxy acids were reported for the 8-17 cm and 17-30 cm sections from a profile of Rostherne Mere recent sediments (Cardoso and Eglinton, 1983). Although the depths are greater in the Rostherne Mere samples, the age of the sections may well correspond closely with those of the 4-8 cm and 8-12 cm sections from Coniston Water (see Livingstone and Cambray, 1978). The small amounts of odd carbon number dicarboxylic acids must have a different origin to simple oxidation of w-hydroxy acids, as no odd carbon number w-hydroxy acids were detected.

4α-Methyl-5α(H)-cholest-8(14)-en-3β-ol present amongst the free lipids of Coniston Water sediment, may originate from a bacterial or an algal source as it has been recognised in a methanotrophic bacterium, Methylococcus capsulatus, (Bird et al., 1970; Bouvier et al., 1976) and in marine dinoflagellates (Kokke et al., 1981), dinoflagellate zooxanthellae (Gagosian et al., 1979) and a freshwater dinoflagellate (Chapter 3, this thesis). The absence of other dinoflagellate sterols (see Robinson et al., 1984a; Chapter 3, this thesis) suggests that methanotrophs are a more likely source in Coniston Water, especially as the lake does not support a large dinoflagellate population.

2.4.ii Lipid diagenesis in Coniston Water sediments

Free lipids in all compound classes decreased in total abundance between the 0-3 cm and 8-12 cm sections, alcohols and ketones were more abundant in the 4-8 cm section than in the 0-3 cm section (Table 2.2/2). In the case of n-alkanes shorter chain compounds were more rapidly removed than longer chain homologues (see Fig. 2.2/2). Unsaturated compounds were not as well preserved as saturated ones, as shown by the detection of free unsaturated alkanoic acids and alkanols only in the 0-3 cm section.

Increasing stanol:stenol ratios are found with increasing sediment depth, presumably due in part to hydrogenation by a bacterial mechanism (Ogura and Hanya, 1973; Gaskell and Eglinton, 1975, 1976) and partly due to preferential degradation of stenols (Nishimura, 1977). In Coniston Water sediment both of the above processes are likely to have contributed to the increase in stanol:stenol ratio with increasing depth, the biggest change occurring between the 0-3 cm section, which includes the oxic zone where bacterial activity and, thus, stenol degradation, is greatest, and the 4-8 cm section.

6,10,14-Trimethylpentadecan-2-one has been suggested as a marker for oxic conditions during sedimentation (Ikan, 1973), but may also be a microbial degradation product of phytol (Brooks and Maxwell, 1974). The source of sedimentary alkan-2-ones is not

clear. Although they resemble n-alkanes in the same sediment in distribution, the correspondence is usually not close enough to substantiate a precursor-product relationship (Cranwell, 1982; Volkman et al., 1983). The lack of a maximum at C_{17} in the free ketones from the sediment of a productive lake, corresponding with the dominance of free C_{17} n-alkane derived from plankton, led Cranwell (1981a) to suggest formation of alkan-2-ones from terrestrially derived n-alkanes, prior to the organic matter reaching the lake. In Coniston Water sediment the alkan-2-ones maximised at C_{27} compared with C_{29} or C_{31} for n-alkanes. Some of the sedimentary alkan-2-ones may have originated from microbiological oxidation of n-alkanes, although no maximum at C_{17} was observed in the 0-3 cm section to correspond with the secondary maximum at C_{17} in the n-alkanes of this section. If microbiological production of alkan-2-ones did take place within the sediment, then the increase in abundance of this compound class between the 0-3 cm and 4-8 cm sections followed by a decrease in the 8-12 cm section, may reflect such production, with degradation being observed after removal from the most active microbial zone.

2.4.iii Comparison of free and bound lipids in Coniston Water sediment

The isolation of free and bound lipids as two separate fractions did not result from incomplete extraction of the free

lipids; furthermore, there was no detectable contamination of one fraction by the other. Evidence to support the above statement includes differences in distribution patterns of free and bound lipids from the same sediment section and the detection of compounds present in one fraction which were absent in the other, e.g. 5β(H)- and 5α(H)-stan-3-ones were present in the free lipid fraction of each section, but were not detected amongst the bound lipids, while only the bound lipid fractions contained steroidal-3,5-dien-7-one compounds.

Differences in the composition of bound lipids compared with free lipids have been interpreted as resulting from a greater contribution of bacterial lipids to the bound fraction of sediments (Brooks et al., 1976; Cranwell, 1978, 1979, 1981a). Coniston Water sediments show this characteristic, having a greater relative abundance of bacterially derived branched acids and alkanols and a higher proportion of shorter chain homologues in the bound lipids than in the free. The relatively greater abundance of short chain homologues in the bound fraction may partly reflect better preservation of bound short chain lipids. Stabilisation of the bound lipids with respect to diagenesis should lead to decreasing free:bound ratios with increasing sediment depth, illustrated for Coniston Water in Fig. 2.4/1. Additionally the increase in total abundance of bound fatty acids with depth (Table 2.2/2) suggests that some in situ formation of bound fatty acids may occur in Coniston Water sediment. Such processes may be dependent upon sedimentary environment. Results published for a marine sediment core from Buzzards Bay, USA, suggest that for that core, bound acids are formed prior to, or

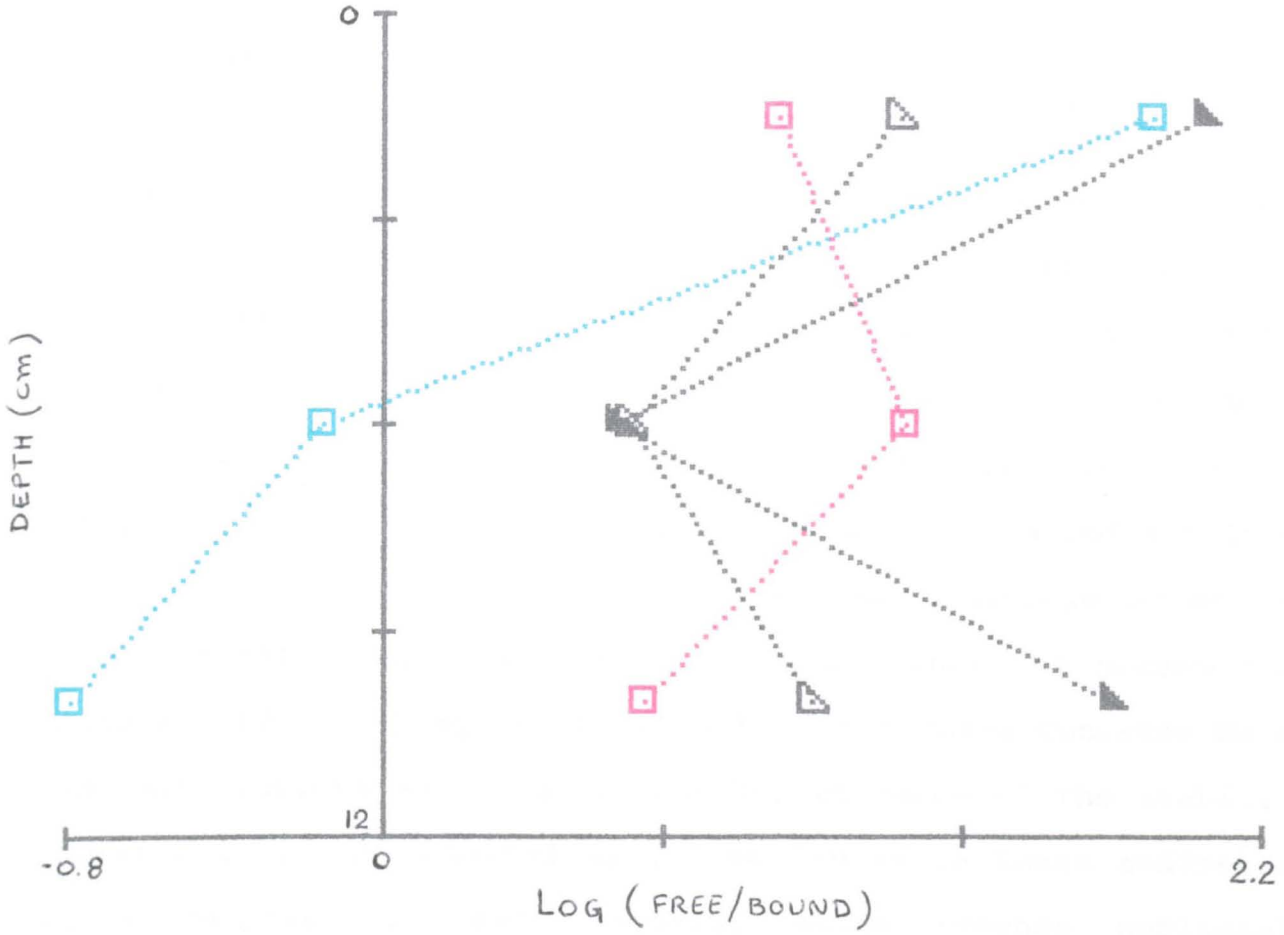


Fig. 2.4/1. Depth profile of ratio of free/bound lipids in Coniston Water sediments.

Key:-

- Hydrocarbons
- Fatty acids
- Alkanols
- Sterols

shortly after deposition and are not stabilised with respect to free acids over the first two hundred years (Farrington et al., 1977); whereas bound acids have been found to be stabilised in another marine sediment (Van Vleet and Quinn, 1979) and in lacustrine sediments (Cranwell, 1981a).

Bound 2- and 3-hydroxy acids in the C_{14}-C_{18} range present in a lacustrine sediment, have been shown to originate from microorganism cell-walls on the basis of stereochemical evidence (Cranwell, 1981b). For the same sediment, stereochemical evidence revealed a different origin, arising from α- and β-oxidation of fatty acids, for free 2- and 3-hydroxy acids and bound 3-hydroxy acids in the range C_{22}-C_{26}. 2-Hydroxy and 3-hydroxy acids are readily metabolisable (Boon et al., 1975). Thus the presence of bound 2- and 3-hydroxy acids in each of the three Coniston Water sediment sections analysed, is further evidence of the stability imparted to bound compared with free lipids in these sediments, in which free 2- and 3-hydroxy acids reached negligible concentrations below the 0-3 cm section. Bound 3-hydroxy acids in Coniston Water sediment reached a minimum abundance in the 4-8 cm section (Fig. 2.2/9). In Rostherne Mere bound 3-hydroxy acids were also least abundant in the middle section of three sediment sections of similar age to those of Coniston Water (Cardoso and Eglinton, 1983; Livingstone and Cambray, 1978).

2.4.iv Elodea nuttallii as a source of sedimentary lipids

A novel tetraterpene alcohol has been reported to occur in E. canadensis (Mangoni et al., 1984). This alcohol was not detected in E. nuttallii collected from Coniston Water, although this may have been due to the low volatility of the compound.

All of the lipids present in E. nuttallii, with the exception of the aldehydes, were recognised in Coniston Water sediments. Aldehydes are likely to be rapidly degraded under oxic conditions. The high n-C_{21} alkane content of E.nuttallii (Fig. 2.3/1) compared with the low concentration of this compound in the sediments (Fig. 2.2/2) argues against a major input of E. nuttallii lipids to the sediments. E. nuttallii is easily uprooted during heavy weather and depending on the water circulation and the bathymetry of the lake, may be concentrated at certain parts of the lake. At such places it is feasible that the input of E. nuttallii lipids may dominate sedimentary lipids arising from other sources.

2.5 CONCLUSIONS

1) Three sections of Coniston Water recent sediments have been analysed for their free and bound lipid contents.

2) A major input of higher plant derived organic matter was

inferred from the distributions of sedimentary lipids, consistent with the known biology of the lake and surrounding areas.

3) The early stages of lipid diagenesis in Coniston Water sediments preferentially remove unsaturated compounds.

4) Free and bound lipids may contain different distributions of lipids, having different origins. A greater microbial input to the bound lipid fraction was recognised.

5) Bound lipids are stabilised relative to the free, inferred from the presence in the bound fractions of labile molecules, which are absent or rapidly removed from the corresponding free fractions and from the decreasing free:bound ratio with increasing sediment depth. Some *in situ* formation of bound lipids may also take place, possibly by conversion of free lipids to a bound state.

6) Comparison of the lipids of *E. nuttallii* with those of the surface sediment demonstrates that *E. nuttallii* is not a major source of the sedimentary lipids of Coniston Water.

CHAPTER THREE

DINOFLAGELLATES AND DINOFLAGELLATE MARKER COMPOUNDS

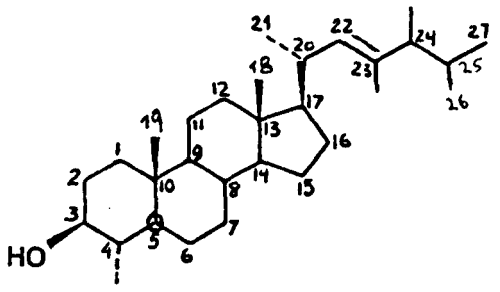
3.1 INTRODUCTION

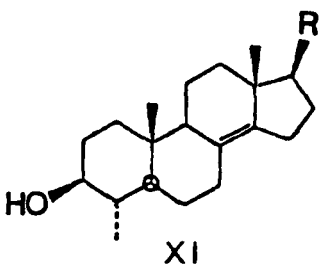
One major aim of organic geochemistry is to be able to reconstruct the environment of deposition of a sediment from its lipid composition, by recognising marker compounds for inputs from specific classes of organisms. A prerequisite for the achievement of this goal is a thorough knowledge of the lipid composition of potential contributor organisms. Although studies have been made to address this problem with respect to marine organisms (e.g., Han et al., 1968; Han and Calvin, 1969; Gelpi et al., 1970; Blumer et al., 1971; Volkman et al., 1980, 1981; Withers, 1983) much still remains to be done.

Steroids are a particularly valuable class of compounds for use as source indicators of organic matter in sediments because of their abundance in organisms, characteristic distributions in different organisms and their relative stability in the sedimentary environment. Sedimentary processes converting the biolipid sterols and steroidal ketones to geolipid steroids have been extensively studied (see Mackenzie et al., 1982; Brassell et al., 1983). The origin and fate of 4-methylsteroids in the environment, however, is less well understood. 4α-Methylsterols and 4α-methylsteroidal ketones have been identified in contemporary and ancient sediments (e.g. Mattern et al., 1970; Boon et al., 1979; Gagosian et al., 1980; Cranwell, 1982; De Leeuw et al., 1983; Brassell and Eglinton, 1983), and 4α-methylsteroidal hydrocarbons occur in ancient sediments and petroleums (e.g. Kimble et al., 1974a; Rubinstein and Albrecht, 1975; Ensminger et al., 1978). 4-Methylsterols are rare in terrestrial organisms,

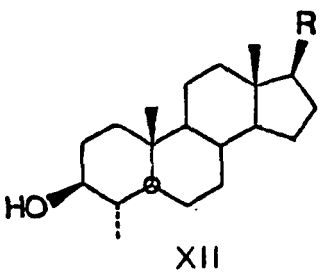
occuring at trace levels in some higher plants and mammalian tissue, for example, they have been reported to occur in the skin of citrus fruits (Mazur et al., 1958; Williams et al., 1967). Prior to 1976 the only 4-methylsterols detected in algae were $\Delta^{8(9)}$ unsaturated 4α-methylsterols which were minor constituents in Porphyridium cruentum (unicellular red alga) (Beastall et al., 1974; Minale and Sodano, 1976) and Euglena gracilis (Beastall et al., 1974). The presence of 4α-methylsterols as significant lipid components was first recognised in the methanotrophic bacterium Methylococcus capsulatus, which contains 4α-methyl-5α(H)-cholest-8(14)-en-3β-ol (Bird et al., 1971; Bouvier et al., 1976). In 1976, dinosterol , 4α,23,24R-trimethyl-5α(H)-cholest-22E-en-3β-ol(XIIi), was identified in the dinoflagellate Gonyaulax tamarensis (Shimizu et al., 1976), and has since been recognised in many other species of marine dinoflagellates, together with a wide variety of other 4α-methylsterols (Fig. 3.1/1).

Dinosterol has been termed a "molecular fossil" for determining past dinoflagellate blooms in the Black Sea (Boon et al., 1979) and, together with other 4α-methylsterols, has been used as an indicator of dinoflagellate inputs to other marine sediments (Brassell and Eglinton, 1983; De Leeuw et al., 1983). The suggestion (Dastillung et al., 1980) that bacteria like M.capsulatus are the source of the dominantly ring saturated 4α-methylsterols in marine sediments seems unlikely as this methanotroph is reported to contain only nuclear unsaturated components (Bird et al., 1971; Bouvier et al., 1976). Previous reports have inferred bacterial de novo synthesis of

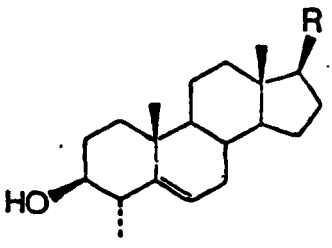




XI



XII



XIII

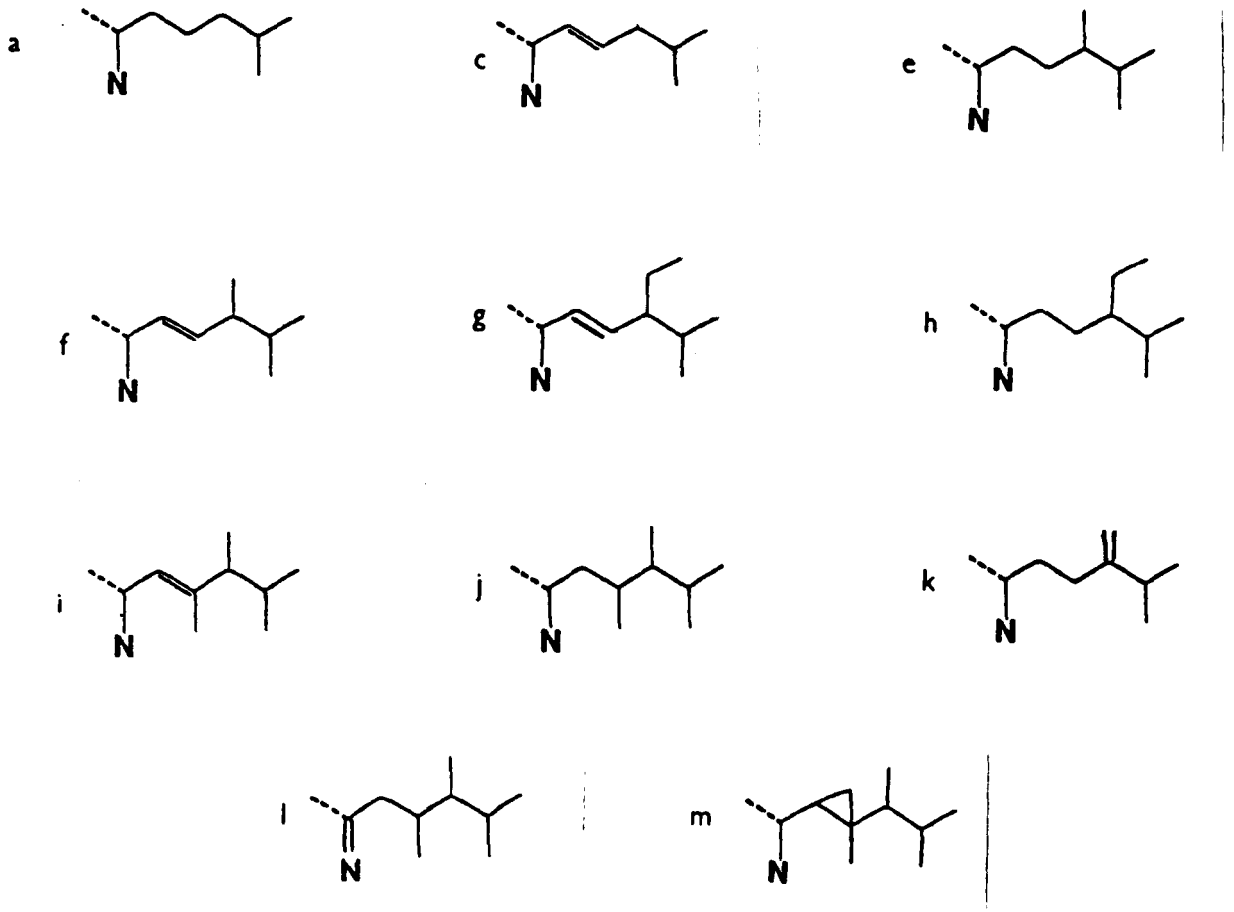


Fig. 3.1/1. Structures of 4α-methylsterols showing numbering sequence and variety found in nature.

4α-methylsterols in lacustrine sediments (Dastillung et al., 1980; Mermoud et al., 1982), based on their occurrence in M. capsulatus and their absence both in potential external sources such as plankton and in the products of sterol incubation in the sediments (Mermoud et al., 1982). Work described in this thesis, however, has shown that, as in the marine situation, dinoflagellates are probably more significant than methanotrophic bacteria as a source of 4α-methylsterols in lacustrine sediments, and, furthermore, can provide a direct input of 5α(H)-stanols and 4α-methylsteroidal ketones (Robinson et al., 1984a). Dinoflagellate blooms may be aggregated both vertically and horizontally (George and Heaney, 1978) and may be short-lived. Hence, plankton sampling at monthly intervals, as made during the study of sterols in Lake Leman (Mermoud et al., 1982), might miss a dinoflagellate bloom and its component 4α-methylsterols. The photosynthetic dinoflagellate Gymnodinium arenicolus has been reported to inhabit interstitial waters of Lake Leman (Dragesco, 1965) and, presumably, would not have been present in the plankton tow samples taken by Mermoud et al. (1982) in their attempt to find an external source for the sedimentary 4α-methylsterols. Alternatively, the plankton population of Lake Leman may have changed in recent years, as 4α-methylsterols were not detected in the upper 3cm of the sediment.

The importance to organic geochemistry of a comprehensive understanding of the lipids produced by dinoflagellates lies not only in the variety of unusual sterols they produce, but in the fact that as primary producers in the marine environment they are surpassed in importance only by the diatoms (Tappan, 1980). Such

an important position in the marine food web, coupled with the many unusual characteristics they possess, makes dinoflagellates also worth studying from economic and biological aspects.

Dinoflagellates are widely distributed in both marine and freshwater environments, occuring in the plankton of freshwater lakes, rivers, bogs and ponds, brackish and sea water lakes, seas and oceans, as well as in snow and ice and in the interstitial waters of the sandy beaches of lakes and seas. A bloom of a species of Gymnodinium was observed under the ice at Lake Baikal (Kozhova, 1959), and red snow and ice covering Lake Davos in the Swiss Alps resulted from a massive accumulation of cysts of Woloszynskia pascheri. Similar red snow due to this species has been reported at Lake Simcoe, Ontario, Canada (Gerrath, 1974).

Dinoflagellates, members of the division Pyrrophyta, are unique in combining the characteristics of both the procaryotes and eucaryotes, and of both plants and animals, some being photosynthetic, whereas others are heterotrophic or phagotrophic, parasitic or endo- or ectosymbionts. A typical dinoflagellate is a free-living photosynthetic, biflagellate organism 30-60 µm in length (Fig. 3.1/2) (although Noctiluca is 1500-2000 µm across). The biflagellate motile stage may alternate with an encysted stage which was formerly thought to result only from conditions unfavourable for growth. Evidence now indicates the encysted stage may be a hypnozygote stage in sexual reproduction, meiosis occuring just prior to or at the time of encystment (Von Stosch, 1973). Dinoflagellates posess an unusually large nucleus (dinokaryon or mesokaryon) which is unique among eucaryotes, other than Euglenids, in that the rounded to rodlike beaded

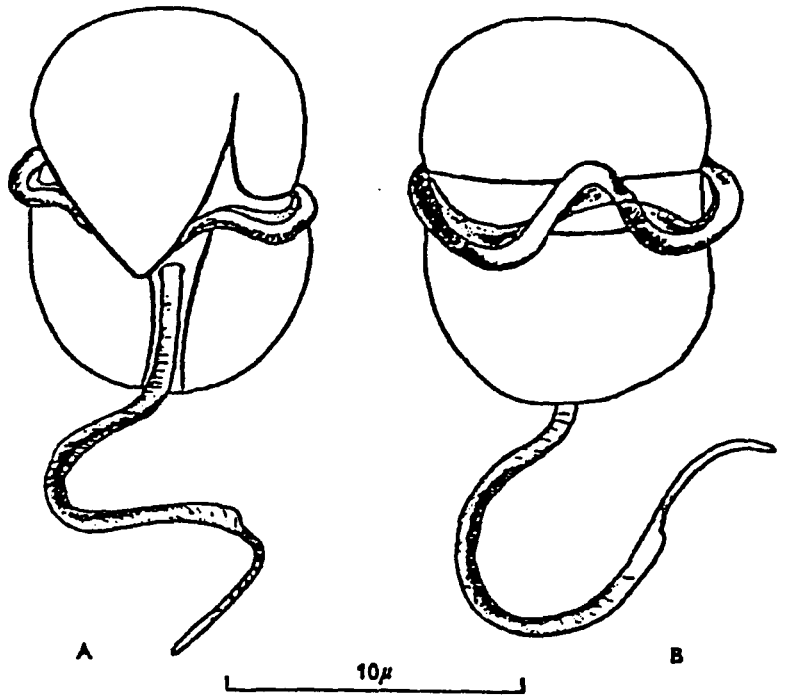


Diagram of Gymnodinium to show the position of the longitudinal and transverse flagella. A, Ventral view of the organism; B, dorsal view of the organism.

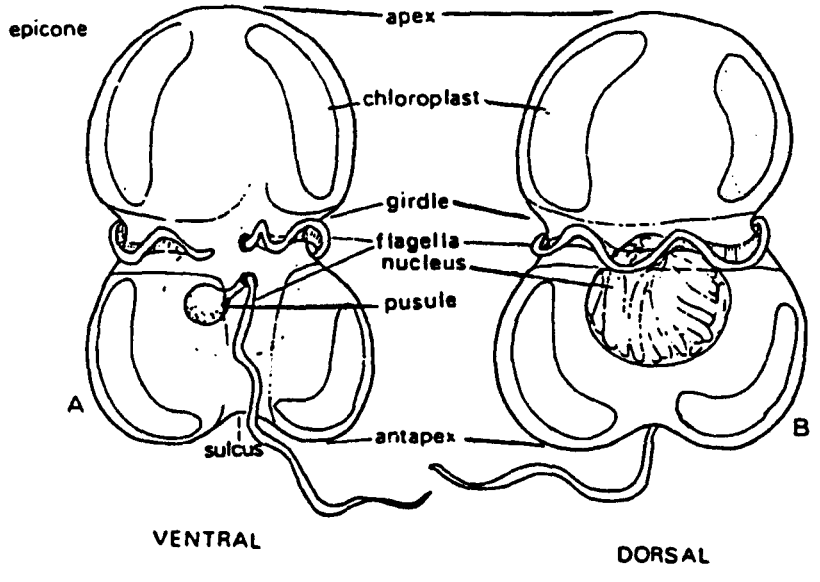


Fig. 3.1/2. Structure of simple dinoflagellate. (Reproduced from Dodge, 1966)

PAGE
MISSING
IN
ORIGINAL

in two respects: the 4α-monomethyl group and the occurrence of an 8(14) double bond. The high degree of biochemical specialisation of dinoflagellates is also evident in their carotenoids. The dominant dinoflagellate carotenoid, peridinin (LXV), has a unique C_{37} (i.e., norcarotenoid) skeleton with an allenic lactone function, and its biosynthesis remains a mystery (Strain et al., 1971). Many other carotenoids are known only from dinoflagellates. A carotenoid/chlorophyll ratio of 3:4 or 3:5 is commonly found (Jeffrey et al., 1975). Both chlorophyll a and chlorophyll c occur, the former generally dominant, but under extreme light conditions or nutrient deficiency chlorophyll c may be dominant.

Only the thick-walled resting cysts, produced by dinoflagellates as a stage in their life history, are fossilised (Evitt, 1961). Following a bloom in Lake Kinneret, Israel, many hundreds of cysts were present per gram of sediment (Tappan, 1980). Some cysts have a resistant organic wall, composed of sporopollenin, but others, such as freshwater Ceratium produce cellulosic cysts which rapidly disintegrate and are not fossilised (Tappan, 1980). Only a few dinoflagellates belonging to the Peridiniales produce acid-resistant cysts. Cysts have been described in detail for only a relatively small number of living dinoflagellates, making it difficult to determine the relationships of many of the fossil taxa that are known solely from such resting stages. The many primitive features of dinoflagellates suggest a long geologic history, but they are best known in the Mesozoic or Cenozoic (Tappan, 1980). Fossils may be present but unrecognised, or else dinoflagellates may have

had a long geolgic history prior to the development of resistant cysts.

The difficulties described above for the study of dinoflagellate fossils, indicate that the molecular record, in the form of specific lipids, may provide a useful record of the palaeobiology of dinoflagellates. In order to achieve this, more information is required on the lipid composition of a variety of dinoflagellate species from a number of different environments. This information may prove useful in the taxonomic classification of dinoflagellates.

In recent years the number of investigations into dinoflagellate lipids has increased dramatically (see Withers, 1983 and references therein). These investigations, however, have concentrated on sterols and have solely involved marine species. This chapter reports on the lipid composition for a number of compound classes of four freshwater species of dinoflagellates: 1) A natural population of Peridinium lomnickii woloszynska, collected from the waters of Priest Pot. U.K. 2) A natural population of P. cinctum fa. westii (Lemm.) Lef., collected from the waters of Lake Kinneret, Israel. 3) A culture of Ceratium hirundinella. 4) A culture of Woloszynskia coronata (Woloszynska) Thompson.

3.2 RESULTS

3.2.i DESCRIPTION OF SAMPLES

a) Peridinium lomnickii Woloszynska occurs, mainly during late winter months, in the plankton of small lakes in Poland, Holland, France and Central Europe (Starmach, 1974). The organism is egg-shaped, with dimensions of 25-40 μm x 22-35 μm. A sample of P. lomnickii was collected from Priest Pot, a eutrophic lake in the English Lake District, at a water depth of 1.2 m when present in high abundance (chlorophyll a >1 mg l^{-1}). Separated lipids of P. lomnickii were supplied by Dr. P.A.Cranwell (Freshwater Biological Association (FBA), U.K.). Lipids were extracted ($CHCl_3$-MeOH) by ultrasonication followed by exhaustive extraction using a Soxhlet apparatus. The total lipid extract was separated by Al_2O_3 column chromatography and SiO_2 TLC (Fig. 3.2/1).

b) P. cinctum fa. westii (Lemm.) Lef. is unique to Lake Kinneret, Israel, where it is the dominant member of the phytoplankton; after an exponential growth phase (November-late March), the Peridinium population remains stable, forming 99% of the total phytoplankton biomass until late April (Serruya, 1978). The organism is subspherical in form, varying in size from 42-66 μm (longitudinal diameter) and from 36-60 μm (transverse diameter) (Serruya, 1978). A natural population, collected (Dr. M.Gophen, Kinneret Limnological Institute) from the north of Lake Kinneret under bloom conditions (20/3/84), was freeze-dried and

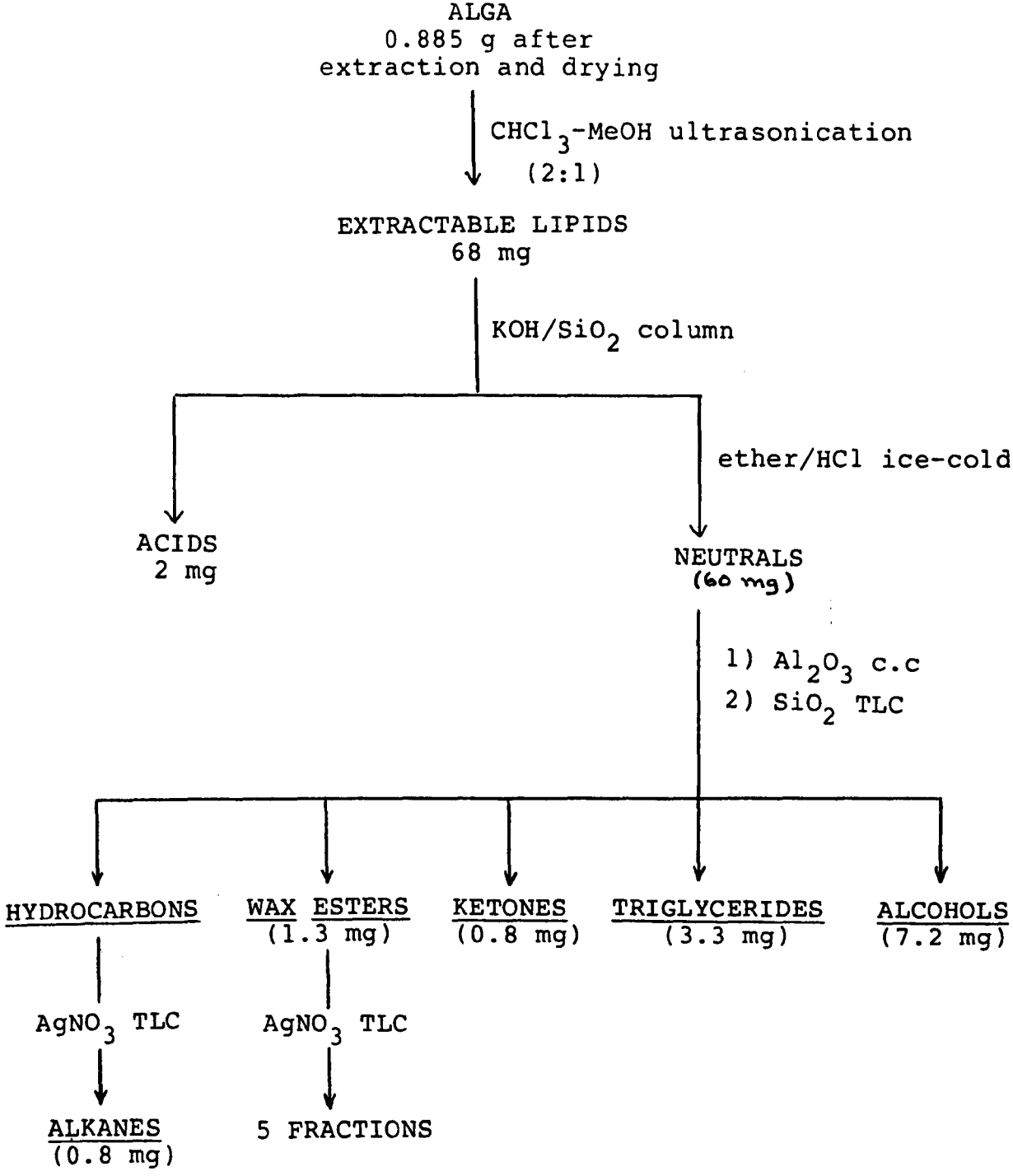


Fig. 3.2/1. Schematic diagram showing the extraction and chromatographic separation of P. lomnickii extractable lipids (see Chapter 8 for experimental details). In addition to the fractions shown, a large amount of polar material was present, which was not further analysed. Compound classes underlined refers to the lipid content of the various fractions as subsequently determined by GC-MS analysis.

sent by airmail to this laboratory. Inspection by optical microscopy and phase contrast microscopy of an aliquot suspended in water revealed P. cinctum to constitute >99% of total phytoplankton, and to contain very low levels of attached bacteria. Extractable lipids were obtained from lyophilised cells (800 mg) with CH_2Cl_2 (ultrasonication). The total lipid extract was separated by Al_2O_3 column chromatography and SiO_2 TLC (Fig. 3.2/2)

c) Ceratium hirundinella is worldwide in distribution, in both brackish and freshwater. It was one of the first two dinoflagellates to be formally described (Muller, 1773). C. hirundinella may be up to 400 µm long; the cyst it produces is cellulosic and is not preserved in sediments. Although C. hirundinella contains many chromoplasts it has a requirement for additional vitamins and may ingest other organisms, thus being myxotrophic; its food vacuoles may contain remains of bacteria (including cyanobacteria) and diatoms, all apparently undergoing digestion (Dodge and Crawford, 1970). Certain of the lipids of C. hirundinella have been analysed by Cranwell (1976) in an investigation into the organic compounds in detritus resulting from microbial attack on the alga. A culture of C. hirundinella, grown by Mr. G.H.M.Jaworski [Freshwater Biological Association (F.B.A.), U.K.], gave, upon harvesting, ca. 3 g wet weight of algae. Extraction by ultrasonication with MeOH-$CHCl_3$ yielded 66 mg lipids. Chromatographic separation as in Fig. 3.2/2 (Al_2O_3 column chromatography, SiO_2 TLC) gave fractions with mobilities corresponding to hydrocarbons, wax esters, ketones, alcohols and fatty acids.

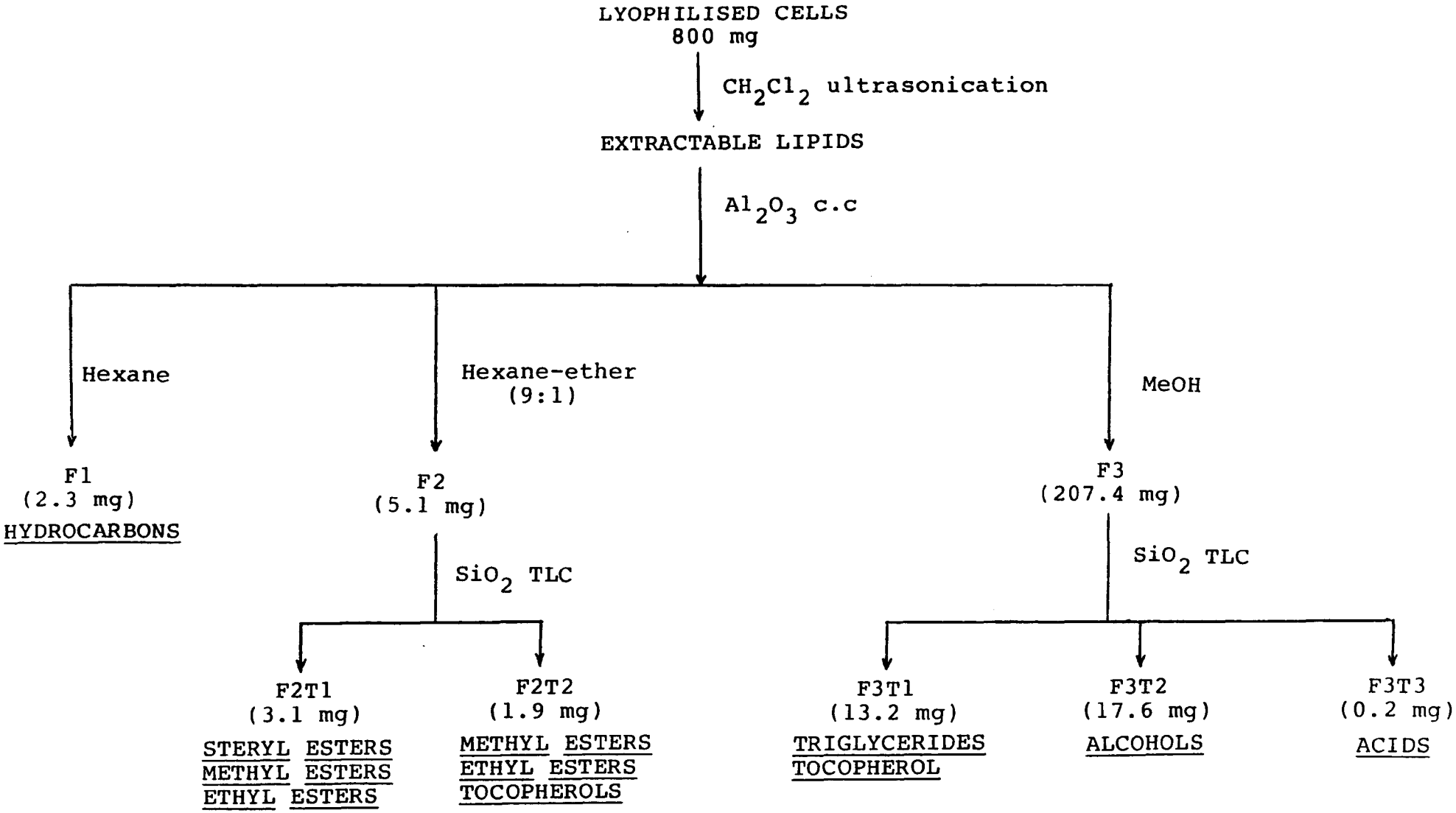


Fig. 3.2/2. Schematic diagram showing extraction and chromatographic separation of P. cinctum extractable lipids (see Chapter 8 for experimental details). In addition to the fractions shown, a large amount of polar material was present, which was not further analysed. Compound classes underlined refers to the lipid content of the various fractions as subsequently determined by GC-MS analysis.

d) Woloszynskia coronata (Woloszynska) Thompson often occurs in small lakes and in clay pits and pools (Starmach, 1974). The organism is almost spherical with dimensions of 19-35 um x 14-32 um. A culture was obtained from the Culture Centre of Algae and Protozoa, Cambridge, U.K., and grown up by Mr. G.H.M.Jaworski (F.B.A., U.K.). Extractable neutral lipids (69 mg) were supplied by Dr. P.A.Cranwell (F.B.A.,U.K.). Chromatographic separation similar to Fig. 3.2/2 (SiO_2 TLC) gave fractions with mobilities corresponding to hydrocarbons, wax esters, ketones, alcohols and "polar material".

3.2.ii. Hydrocarbons

The hydrocarbons of P. lomnickii consisted mainly of n-alkanes ranging from C_{15}-C_{33}, maximising at C_{17} and C_{27} and with a CPI close to 1. Each of the other three dinoflagellate species had a very low n-alkane content showing no odd carbon predominance, but contained high levels of heneicosahexaene ($C_{21:6}$). Low levels of squalene were detected in each case. P. cinctum was also found to contain unknown polyenes with KRI values of ca. 2580 and ca. 3600.

3.2.iii. Esters

a) Wax and steryl esters

A series of straight chain wax esters (C_{34}-C_{46}) was isolated from P. lomnickii. Each of the four dinoflagellates studied contained steryl esters. Analyses were made by GC and GCMS using a 15 m x 0.32 mm flexsil column (phase thickness 0.1 um) and on column injection. Identifications were made by mass spectral interpretation based on the mass spectra of steryl ester standards (Fig. 3.2/3), and, in the case of P. lomnickii, Ag^+ TLC mobility; the EI mass spectra were found to show ions consistent with cleavage of the ester bond and retention of charge by steryl fragments. The fractions containing steryl esters were also found to contain esters of phytol and, in one case, dihydrophytol. The steryl and phytyl esters identified in the organisms are listed in Table 3.2/1 with an indication of their relative abundance. Some mass fragmentograms useful in identifying the steryl esters of W. coronata are shown in Fig. 3.2/4.

b) Methyl and ethyl esters

A series of methyl esters and a series of ethyl esters were detected in P. cinctum, C. hirundinella and W. coronata (Fig. 3.2/5). Ethanol was not used in the preparation of any of the lipid fractions, methanol was not used in the isolation of methyl and ethyl esters from P. cinctum. The identity of the C_{16} fatty acid ethyl ester was confirmed by cochromatography with a

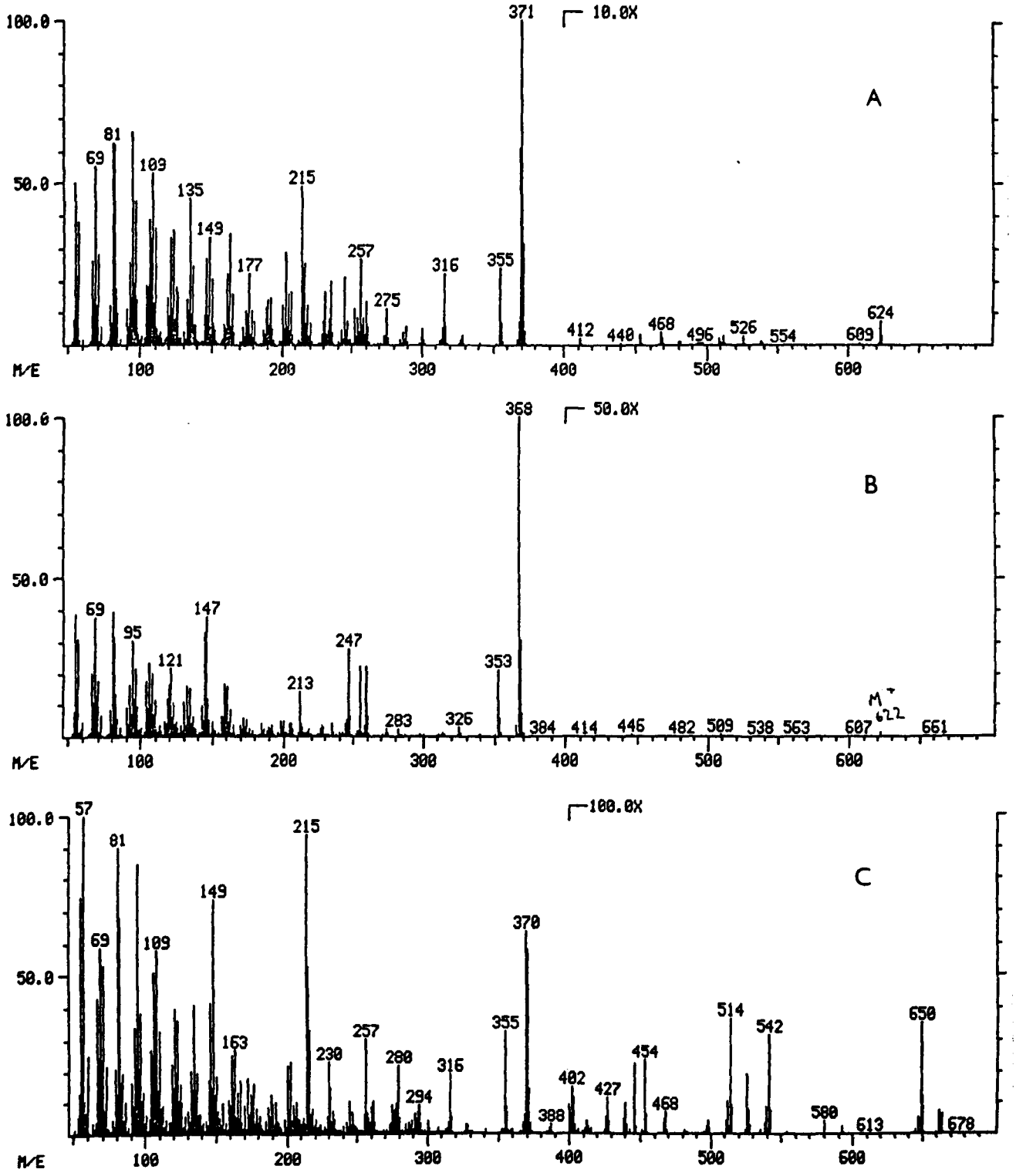


Fig. 3.2/3. EI mass spectra of steryl ester standards (purchased from Nucheck Prep, USA).
A=cholestanol-16:1; B=cholesterol-16:1; C=cholestanol-18:2
Spectra obtained by direct insert probe MS using a Finnigan 4000 mass spectrometer. Operating conditions as described in Chapter 8.

Table 3.2.1 Steryl and phytyl esters identified in the dinoflagellates P. lomnickii, P. cinctum, C. hirundinella and W. coranata

Compound[a]	Abundance[b]			
	P.l.	P.c.[c]	C.h.[a]	W.c.
Desmethylsteryl esters				
C_{27} Δ^5 - 14:0	tr.	++	+	N.D.
C_{27} - 14:0	++	+	tr.	N.D.
C_{27} Δ^5 - 16:0	tr.	+++	+++	N.D.
C_{27} - 16:0	++	++	++	N.D.
C_{27} Δ^5 - 18:1	N.D.	+++	+++	N.D.
C_{27} - 18:1	++	+	++	N.D.
C_{27} Δ^5 - 18:0	tr.	+	N.D.	N.D.
C_{27} - 18:0	+	tr.	N.D.	N.D.
C_{27} - 20:1	tr.	N.D.	N.D.	N.D.
4-Methylsteryl esters				
4,Me C_{28} - 14:0	+	+	N.D.	+
4,24-di Me Δ^{22} - 14:0	N.D.	tr.	N.D.	N.D.
4,24-di Me - 14:0	+	+	N.D.	+
4,23,24-tri Me $\Delta^{5,22}$ - 14:0	N.D.	+	N.D.	+
4,23,24-tri Me Δ^{22} - 14:0	+	tr.	N.D.	N.D.
4,23,24-tri Me - 14:0	+	N.D.	N.D.	N.D.
4-Me C_{28} - 16:0	++	+	N.D.	+++
4,24-di Me Δ^{22} - 16:0	tr.	tr.	N.D.	N.D.
4,24-di Me - 16:0	+	+	N.D.	++
4,23,24-tri Me $\Delta^{5,22}$ - 16:0	++	+	N.D.	++
4,23,24-tri Me Δ^{22} - 16:0	++	tr.	N.D.	N.D.
4,23,24-tri Me - 16:0	+	N.D.	N.D.	N.D.
4-Me C_{28} - 18:1	++	+	N.D.	+
4,24-di Me Δ^{22} - 18:1	N.D.	tr.	N.D.	N.D.
4,24-di Me - 18:1	N.D.	+	N.D.	+
4,23,24-tri Me $\Delta^{5,22}$ - 18:1	N.D.	++	N.D.	+++
4,23,24-tri Me Δ^{22} - 18:1	N.D.	tr.	N.D.	N.D.
4,23,24-tri Me - 18:1	+	N.D.	N.D.	N.D.
4-Me C_{28} - 18:0	tr.	N.D.	N.D.	N.D.
4-Me C_{28} - 20:1	tr.	N.D.	N.D.	N.D.
Phytyl esters[e]				
Phytol - 14:0	++	+	N.D.	N.D.
Phytol - 16:0	+++	++	N.D.	N.D.
Dihydrophytol - 18:0[f]	N.D.	++	N.D.	N.D.
Phytol - 18:1[f]	++	+	N.D.	N.D.
Phytol - 18:0	++	N.D.	N.D.	N.D.
Phytol - 20:0	+	N.D.	N.D.	N.D.
Phytol - 22:0	+	N.D.	N.D.	N.D.
Phytol - 24:0	+	N.D.	N.D.	N.D.

(a) Compounds are written in a shorthand notation in the form alkyl-acyl, e.g. 4,23,24-tri Me $\Delta^{5,22}$ - 18:1 refers to 4,23,24-trimethylcholesta-5,22-dien-3β-ol esterified with a monounsaturated C_{18} carboxylic acid. Identifications were made by comparison of mass spectra with those of standards (see text). The data was complex with many occurrences of overlapping peaks, hence, some minor components may not have been recognised.

(b) Abundances were classified on the following basis:-

+++ Major or predominant component
++ 10 - 15% of most abundant component
+ 1 - 10% of most abundant component
tr. Trace levels present
N.D. Not detected

(c) Unidentified 4-methylsteryl esters with shorter acyl chains were present

(d) Unidentified minor steryl ester components were present.

(e) Identifications made by mass spectral interpretation ($M^{\cdot+}$, m/z 278, 123, 95, 82,68).

(f) Tentative assignment based on spectral interpretation and relative retention time.

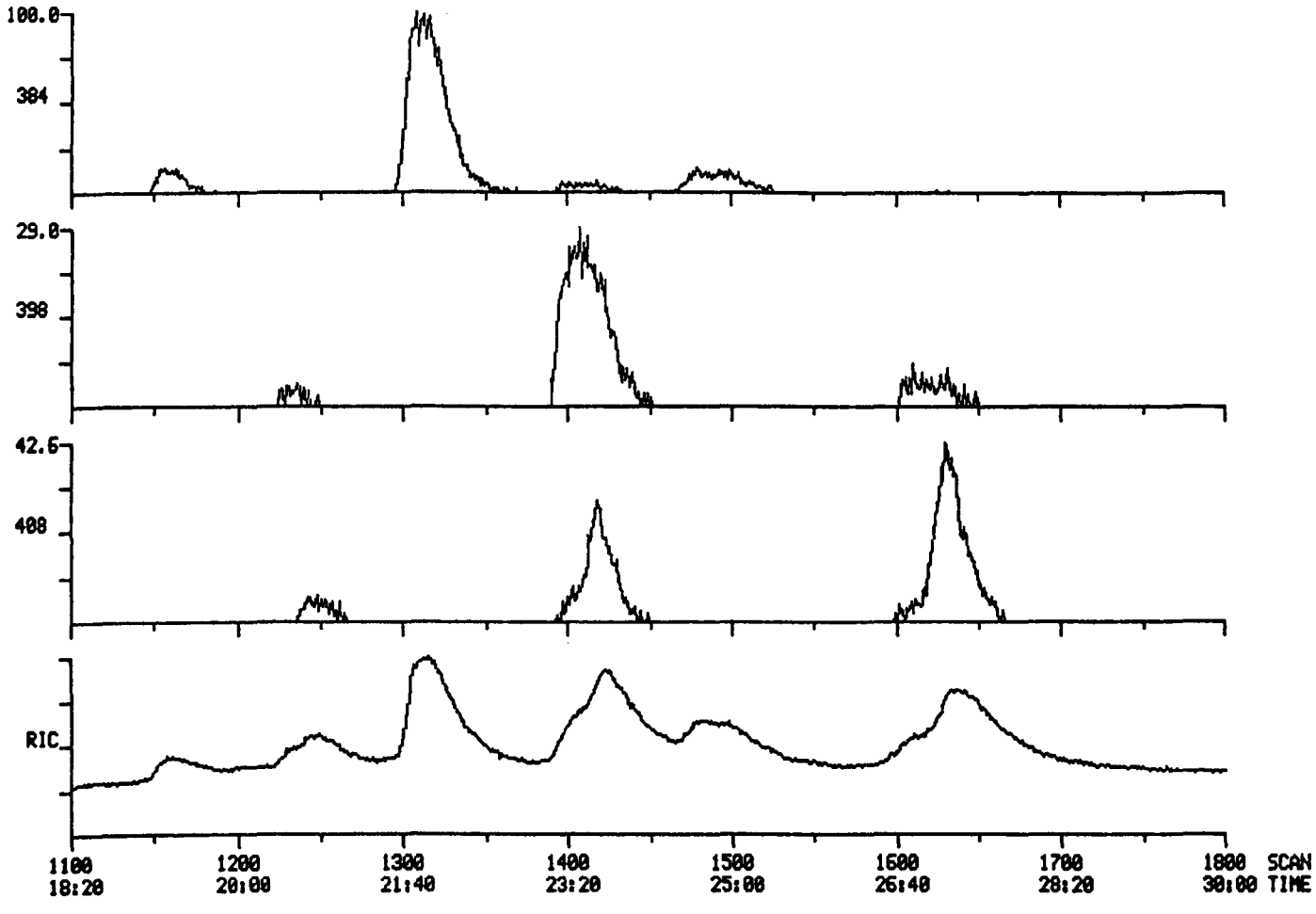


Fig. 3.2/4. Partial RIC and m/z 384, m/z 398 and m/z 408 mass fragmentograms showing the distribution of steryl esters in W. coronata. The above ions represent $C_{28:0}$, $C_{29:0}$ and $C_{30:2}$ steryl fragments. Full identifications, made by spectral interpretation based on the mass spectra of steryl ester standards (Fig. 3.2/3), and approximate abundances are given in Table 3.2/1.

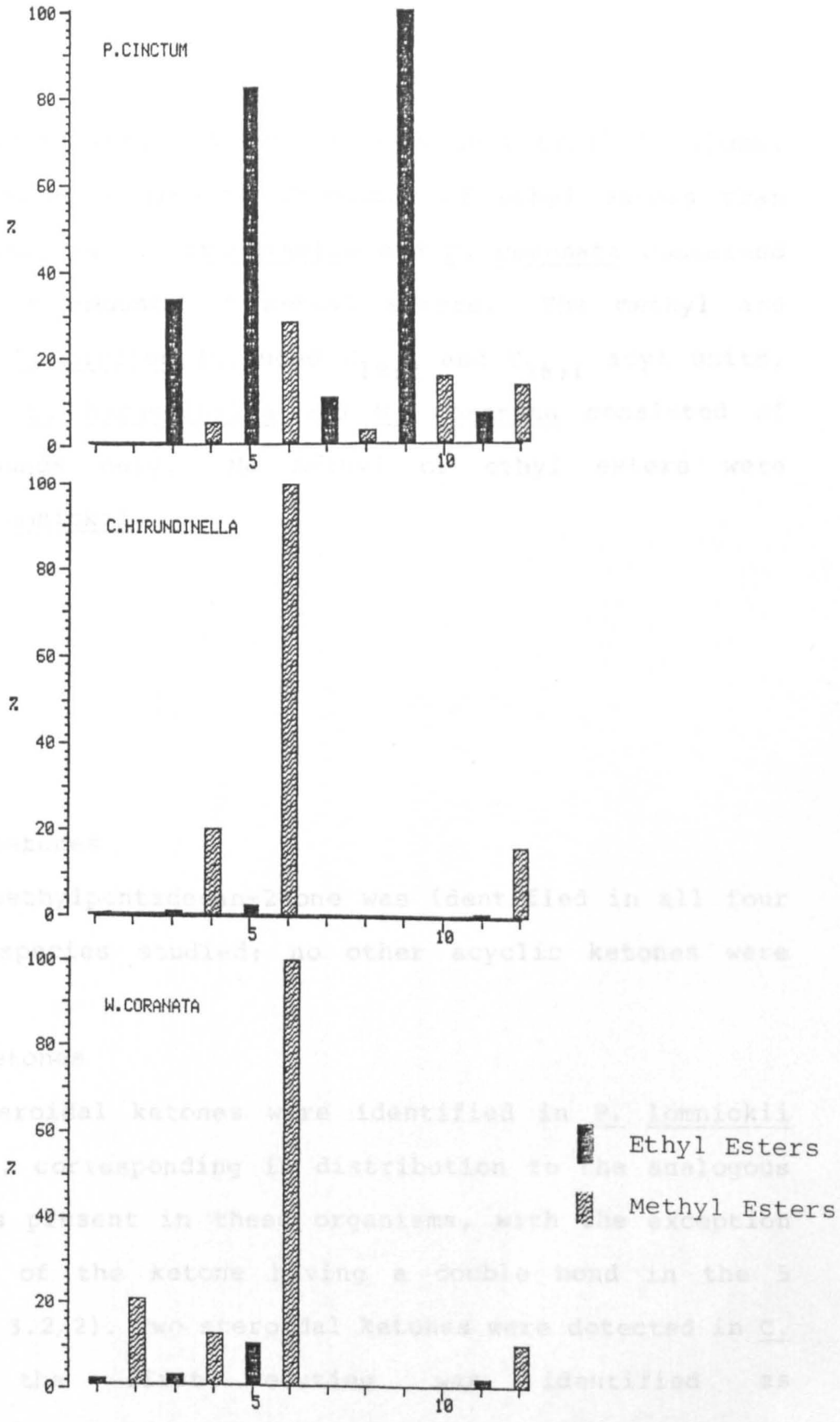


Fig. 3.2/5. Distribution of methyl and ethyl esters in P. cinctum, C. hirundinella and W. coronata.
Key:-
1=C_{12} FAEE 2=C_{12} FAME 3=C_{14} FAEE 4=C_{14} FAME
5=C_{16} FAEE 6=C_{16} FAME 7=$C_{18:2}$ FAEE 8=$C_{18:2}$ FAME
9=$C_{18:1}$ FAEE 10=$C_{18:1}$ FAME 11=C_{18} FAEE 12=C_{18} FAME
(FAEE=fatty acid ethyl ester, FAME=fatty acid methyl ester)

synthesised standard (see Chapter 8) on a 50 m CPSil 5 column. P. cinctum contained a greater abundance of ethyl esters than methyl esters, whereas C. hirundinella and W. coronata contained relatively greater amounts of methyl esters. The methyl and ethyl esters of P. cinctum included $C_{18:2}$ and $C_{18:1}$ acyl units, whilst those of C. hirundinella and W. coronata consisted of saturated compounds only. No methyl or ethyl esters were detected in P. lomnickii.

3.2.iv Ketones

a) Acyclic ketones

6,10,14-Trimethylpentadecan-2-one was identified in all four dinoflagellate species studied; no other acyclic ketones were detected.

b) Cyclic ketones

4α-Methylsteroidal ketones were identified in P. lomnickii and W. coronata, corresponding in distribution to the analogous 4α-methylsterols present in these organisms, with the exception of the absence of the ketone having a double bond in the 5 position (Table 3.2/2). Two steroidal ketones were detected in C. hirundinella; the first eluting was identified as 5α(H)-cholestan-3-one from its mass spectrum and by cochromatography with an authentic standard, the second eluting compound, present in three times the abundance of the first, was identified as cholest-4-en-3-one from its mass spectrum and by

Table 3.2/2 Steroidal ketones of P. lomnickii and W. coranata

C no.[a]	Compound	Structure	Abundance[b]	
			P.l.	W.c.
28	4α-Methyl-5α(H)-cholestan-3-one	XVI a	70	100
29	4α,24-Dimethyl-5α(H)-cholest-22-en-3-one	XVI f	30	N.D.
29	4α,24-Dimethyl-5α(H)-cholestan-3-one	XVI g	35	91
30	4α,23,24-Trimethyl-5α(H)-cholest-22-en-3-one	XVI i	100	35
30	4α,23,24-Trimethyl-5α(H)-cholestan-3-one	XVI j	40	N.D.

(a) Number of carbon atoms

(b) Abundances given as % most abundant component. Identifications made by comparison of mass spectrum with a reference spectrum and/or with published data (e.g. Brassell, 1980; Djerassi, 1978; Gagosian and Smith, 1979; Wardroper, 1979) or, where not available, mass spectral interpretation and relative retention time

(N.D.) Not detected

cochromatography with an authentic standard. P. cinctum contained no detectable cyclic ketones.

c) Tocopherols

Two non-ketonic compounds were identified in the lipid fraction of P. cinctum which have the same TLC mobility as cholestan-3-one, used as a TLC standard in the isolation of ketones; one was identified as α-tocopherol and the other (ca. 3x as abundant) was identified as the methyl ether of α-tocopherol (Fig. 3.2/6).

3.2.v Alcohols

a) Alkanols

Low levels of C_{16} and C_{18} n-alkanols were detected in the four species of dinoflagellates studied. Phytol was present in each case, being abundant in P. cinctum and C. hirundinella. An additional compound was detected in P. lomnickii with a mass spectrum corresponding to a $C_{20:1}$ diol (TMS) (Fig. 3.2/7). Its relative retention time and ions at m/z 183 and m/z 143 suggested it might resemble phytol but have an extra hydroxyl group. The position of the second hydroxy group could not be determined, some possibilities are indicated in Fig.3.2/7.

b) Sterols

Sterols were major lipid components of each of the four species of dinoflagellates studied, e.g., cholesterol = 3 mg/g dry weight in P. cinctum. C. hirundinella contained only

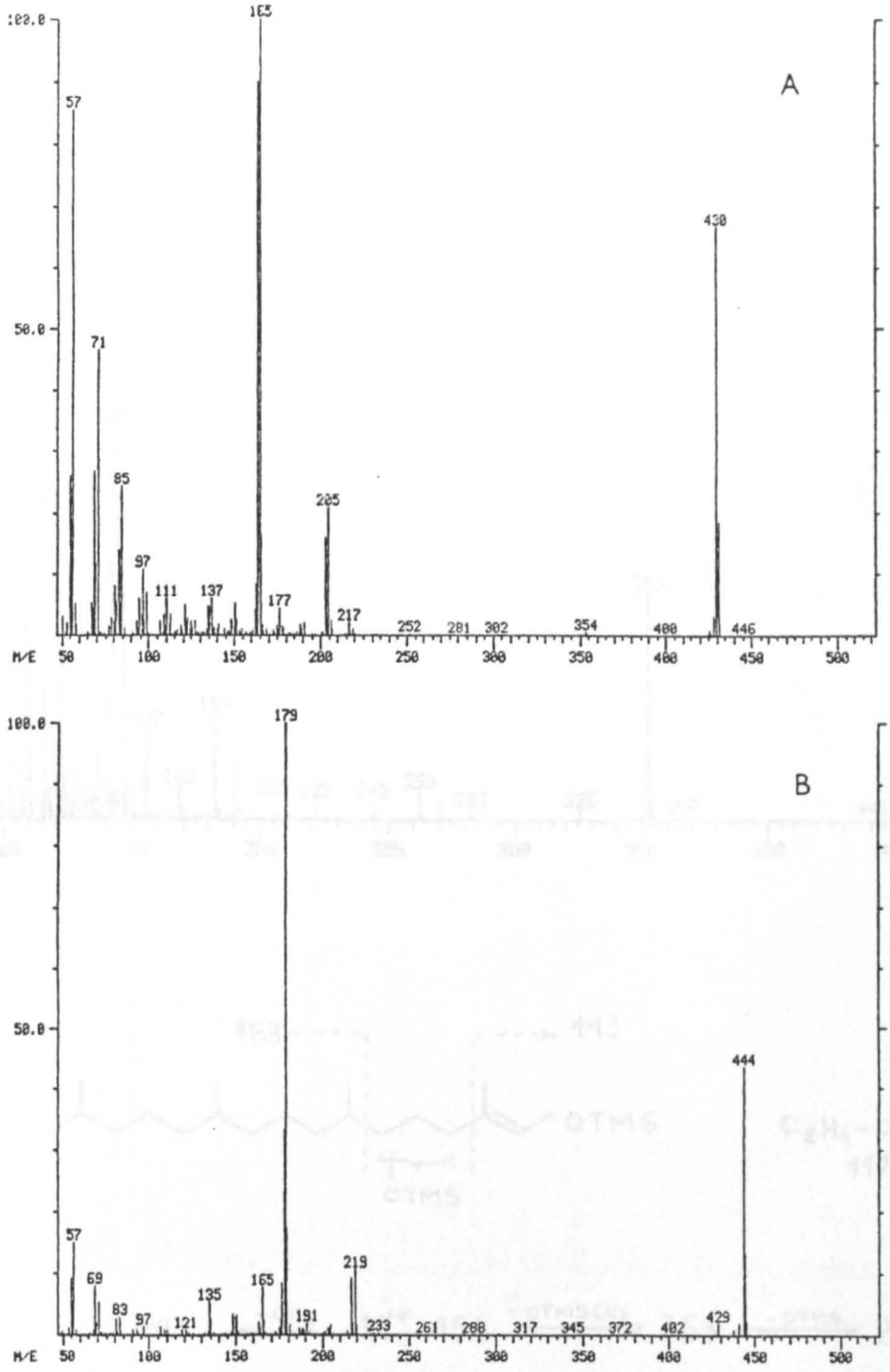
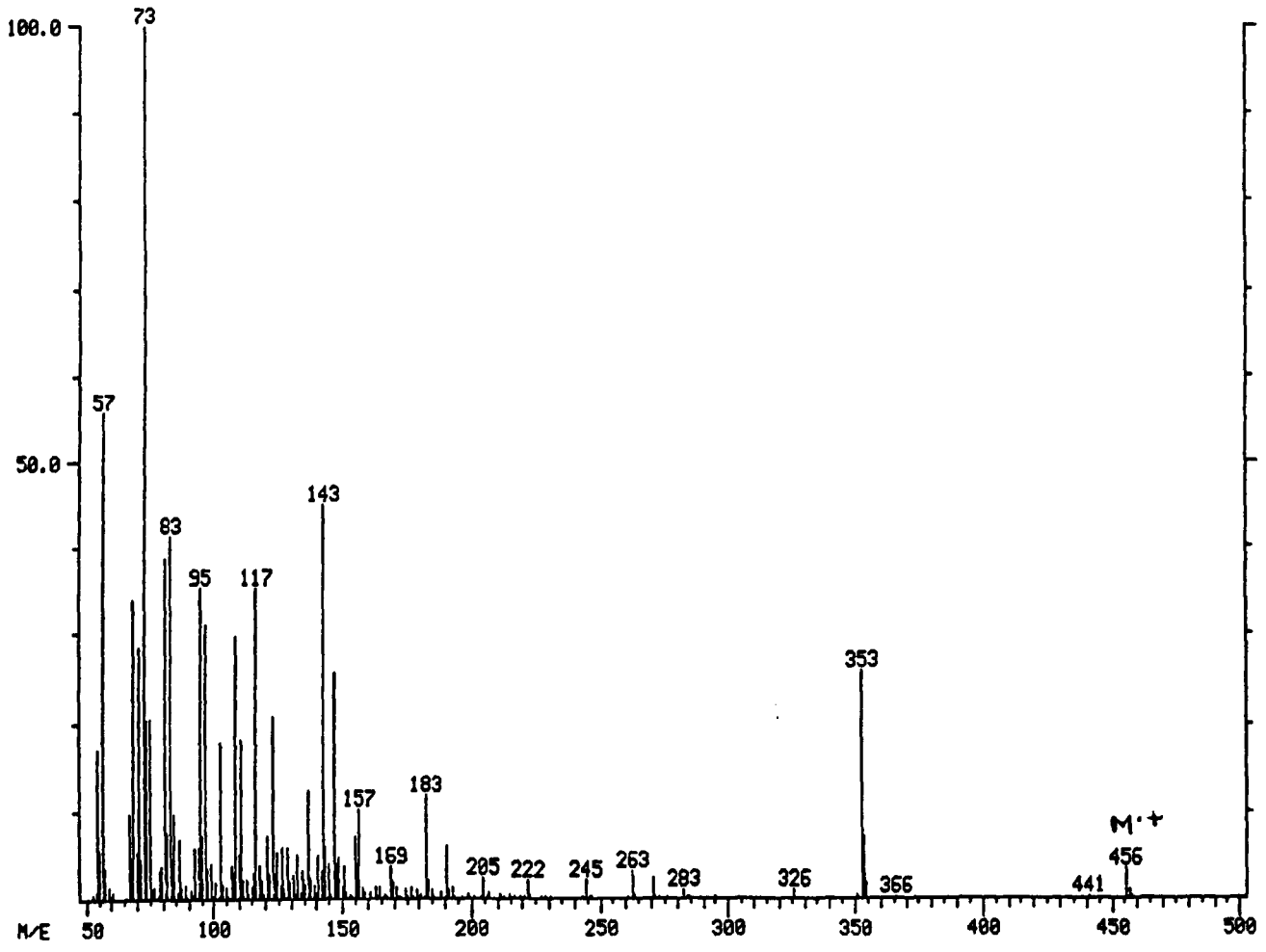


Fig. 3.2/6. Mass spectra from GC-MS of P. cinctum extract of: A α-tocopherol (LXIV) and B methyl ether of α-tocopherol; identified by mass spectral interpretation.



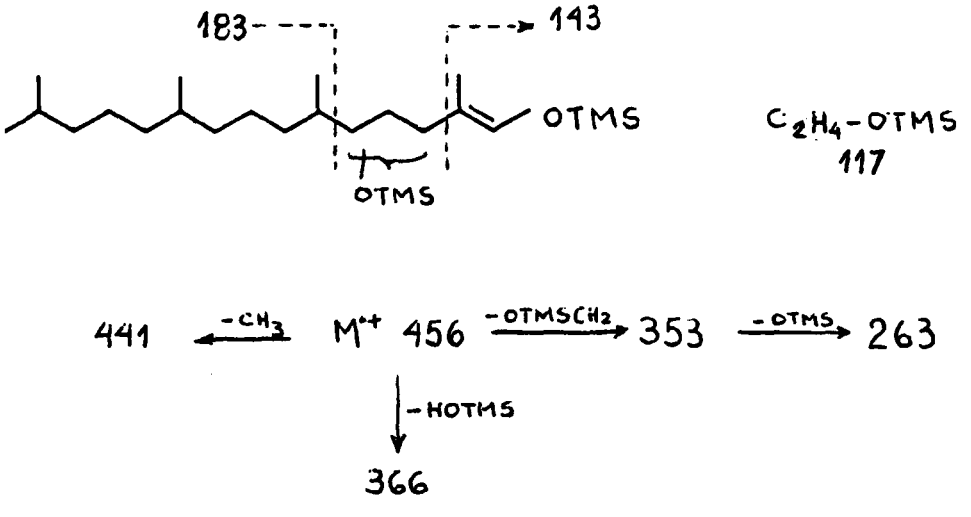


Fig. 3.2/7. Mass spectrum of unknown $C_{20:1}$ diol (TMS) isolated from P. lomnickii. Compound elutes between C_{19} and C_{20} n-alkanols (TMS).

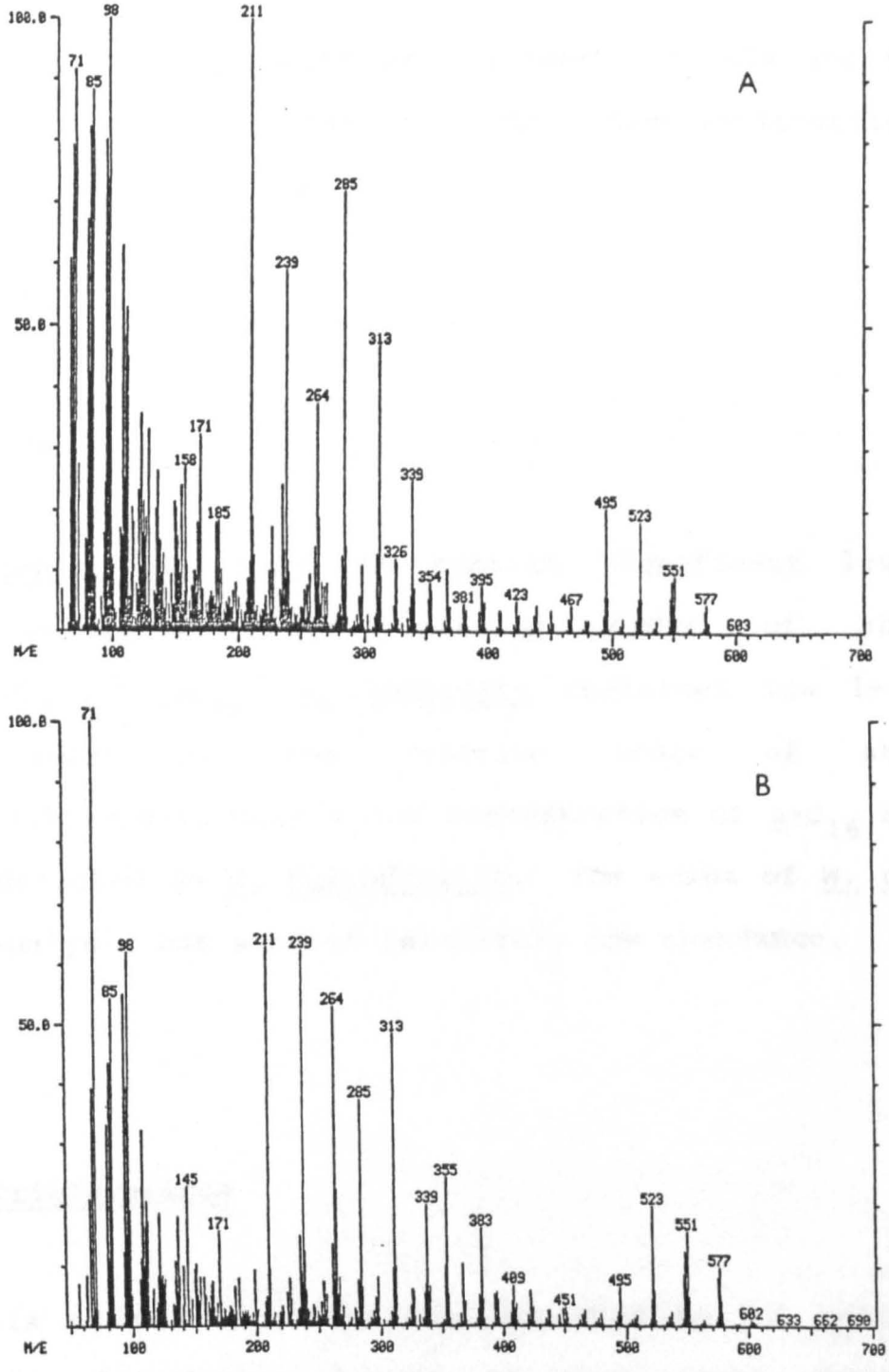


Fig. 3.2/8. Mass spectra of triglycerides of: A P. lomnickii and B W. coronata. Spectra obtained by direct insertion EI probe on Finnigan 4000 mass spectrometer of lipid fractions having the chromatographic mobility of triglycerides. Identifications made by comparison with published data (Wakeham and Frew, 1983).

desmethylsterols, W. coronata only 4α-methylsterols and the two Peridinium species contained both desmethylsterols and 4α-methylsterols (Table 3.2/3).

3.2.vi Acids

P. cinctum was found to contain significant levels of alkanoic acids in the relative order of abundance $C_{18:1}>C_{16}>C_{18:2}>C_{14}>C_{18}$. P. lomnickii contained low levels of alkanoic acids in the relative order of abundance 16:0>>18:0>18:1>14:0. Only a low concentration of n-C_{16} alkanoic acic was detected in C. hirundinella. The acids of W. coronata were not analysed but were of relatively low abundance.

3.2.vii Triglycerides

Analysis of the fractions corresponding in TLC mobility to triglycerides by direct insert EI probe mass spectrometry revealed that each of the four dinoflagellate species studied contained triglycerides, formed mainly from C_{14}, C_{16} and $C_{18:1}$ acyl units (Fig.3.2/6). Ions at m/z 355 and m/z 383 in Fig. 3.2/6B correspond to C_{21} and C_{23} $(M-RCO_2)^+$ fragments. GC-MS analysis of the triglyceride fraction of W. coronata showed 5 relatively early eluting triglycerides each containing one C_4

Table 3.2/3 Sterols of the dinoflagellates P. lomnickii, P. cinctum, C. hirundinella and W. coronata

C no.[a]	Compound[b]	Structure	Abundance[c] P.l.	P.c.	C.h.	W.c.
27	Cholesta-5,22-dien-3β-ol	VI c	-	-	0.3	-
27	5α(H)-Cholest-22-en-3β-ol	III c	-		3.8	-
27	Cholest-5-en-3β-ol	VI a	1	100	100	-
27	5α(H)-Cholestan-3β-ol	III a	100	4	24	-
28	$C_{28:2}$		-	tr.	-	-
28	C_{28} Δ^{22}		-	-	1.1	-
27	5α(H)-Cholest-7-en-3β-ol	VIII a	6	-	-	-
28	24-Methyl-5α(H)-cholest-22-en-3β-ol	III f	-	-	0.6	-
28	4α-Methyl-5α(H)-cholest-8(14)-en-3β-ol	XI a	-	1	-	-
28	24-Methyl-5α(H)-cholest-24(28)-en-3β-ol	III k	-	-	0.8	-
28	24-Methylcholest-5-en-3β-ol	VI e	-	3	-	-
28	4α-Methyl-5α(H)-cholestan-3β-ol	XII a	86	4	-	100
28	24-Methyl-5α(H)-cholestan-3β-ol	III e	3	-	1.5	-
29	4α,24-Dimethyl-5α(H)-cholest-22-en-3β-ol	XII f	15	2	-	-
29	23,24-Dimethyl-5α(H)-cholest-22-en-3β-ol	III i	-	-	0.4	-
29	24-Ethylcholesta-5,22-dien-3β-ol	VI g	-	-	0.2	-
29	24-Ethyl-5α(H)-cholest-22-en-3β-ol	III g	-	-	0.4	-
29	4-Me $C_{29:1}$		-	1	-	-
29	24-Ethylcholest-5-en-3β-ol	VI h	25	-	-	-
29	24-Ethyl-5α(H)-cholestan-3β-ol	III h	45	-	-	-
29	4α,24-Dimethyl-5α(H)-cholestan-3β-ol	XII e		6	-	48
30	4α,23,24-Trimethylcholesta-5,22-dien-3β-ol	XIII i	62	20	-	94
30	4α,23,24-Trimethyl-5α(H)-cholest-22-en-3β-ol	XII i	90	26	-	8
30	4α,23,24-Trimethyl-5α(H)-cholest-17(20)-en-3β-ol[d]	XII l	-	27	-	-
30	4α,23,24-Trimethyl-5α(H)-cholestan-3β-ol	XII j	64	tr.	-	-
30	22,23-Methylene-23,24-dimethylcholest-5-en-3β-ol	VI m	-	-	6.3	-
30	22,23-Methylene-23,24-dimethyl-5α(H)-cholestan-3β-ol	III m	-	-	0.8	-

(a) Number of carbon atoms

(b) Identifications made by combination of comparison of mass spectra with those of standards (e.g. Brooks et al., 1968; Boon et al., 1979), mass spectral interpretation, relative retention times and behaviour after hydrogenation (e.g. the compound identified as 4α-methyl-5α(H)-cholest-8(14)-en-3β-ol was unchanged by the hydrogenation due to the hindered nature of the double bond; a Δ^7 double bond would have migrated to the 8(14) position during hydrogenation, but as no change in relative retention time was observed the double bond position was assigned to the 8(14) position).

(c) Abundances are given as % of most abundant component.

(d) Assignment initially based solely on mass spectral interpretation, but subsequently confirmed by cochromatography with a standard (donated by Dr. W.C.M.C. Kokke).

(-) Not detected.

acyl unit and with the other acyls being composed of all of the possible pairings of C_{14},C_{16} and $C_{18:1}$, with the exception of $C_{18:1}$,$C_{18:1}$ which did not elute under the GC conditions used. The spectra of these compounds contained a large m/z 71 ion, corresponding to C_3H_7CO, which is relatively more intense in Fig. 3.2/6B than in Fig. 3.2/6A, although GC-MS analysis of the triglyceride fraction of *P. lomnickii* did suggest the presence of small amounts of triglycerides containing a C_4 acyl.

3.3 DISCUSSION

3.3.i Steroids

All of the sterols identified in *P. lomnickii*, *P. cinctum*, *C. hirundinella* and *W. coronata* have been previously identified in marine dinoflagellates (Withers, 1983 and references therein), but for many of the sterols this is the first report of their occurrence in freshwater algae. Some of the sterols identified in this study have only been detected in a limited number of dinoflagellate species. Dinostanol, the saturated analogue of dinosterol, has been reported to occur as a minor sterol in the marine dinoflagellate *Gonyaulax diagensis* and as a major sterol in *G. polygramma* and *Gymnodinium wilczeki* (Alam *et al.*, 1978; Volkman *et al.*, 1984; Nichols *et al.*, 1984). 4α,23,24-Trimethylcholesta-5,22-dien-3β-ol was isolated as a major sterol from the heterotrophic dinoflagellate

Crypthecodinium cohnii (Withers et al., 1978) and has been subsequently detected as a trace sterol in several other species (Withers, 1983). 4α,23R,24R-Trimethylcholest-17(20)-en-3β-ol, peridinosterol, is a rare sterol which has been isolated from the two binucleate species P. foliaceum and Exuviaella mariae-lebourae (Withers et al., 1979a; Swenson et al., 1980; Withers, 1983).

The large variety of sterol structures found in dinoflagellates has prompted interest in their mechanisms of biosynthesis. Usually the major sterols of dinoflagellates are 4α-methyl ring saturated compounds with additional methyl groups at C-23 and C-24 of the side-chain (e.g., dinosterol), but the abundant desmethylsterols are often unsaturated and have unsubstituted side-chains (e.g., cholesterol). This has been taken to indicate that there is a dichotomy in dinoflagellate sterol biosynthetic pathways, one branch apparently leading to the 4α-methylsterols and the other route, in which side-chain alkylation is relatively unimportant, leading to the 4-desmethylsterols (Withers et al., 1978).

Biosynthetic studies involving axenic cultivation of the heterotrophic species C.cohnii in a nutrient medium supplemented with CD_3-labelled methionine, led to a proposed mechanism for dinosterol and gorgosterol production as shown in Fig. 3.3/1 (Withers et al., 1979b). An alternative pathway has been proposed whereby bioalkylation of an isolated side-chain double bond occurs, even without the presence of a 24-methyl substituent, giving rise to a 23-methyl-Δ^{22}-sterol (Djerassi, 1981). Support for the latter mechanism occurring in some dinoflagellates has

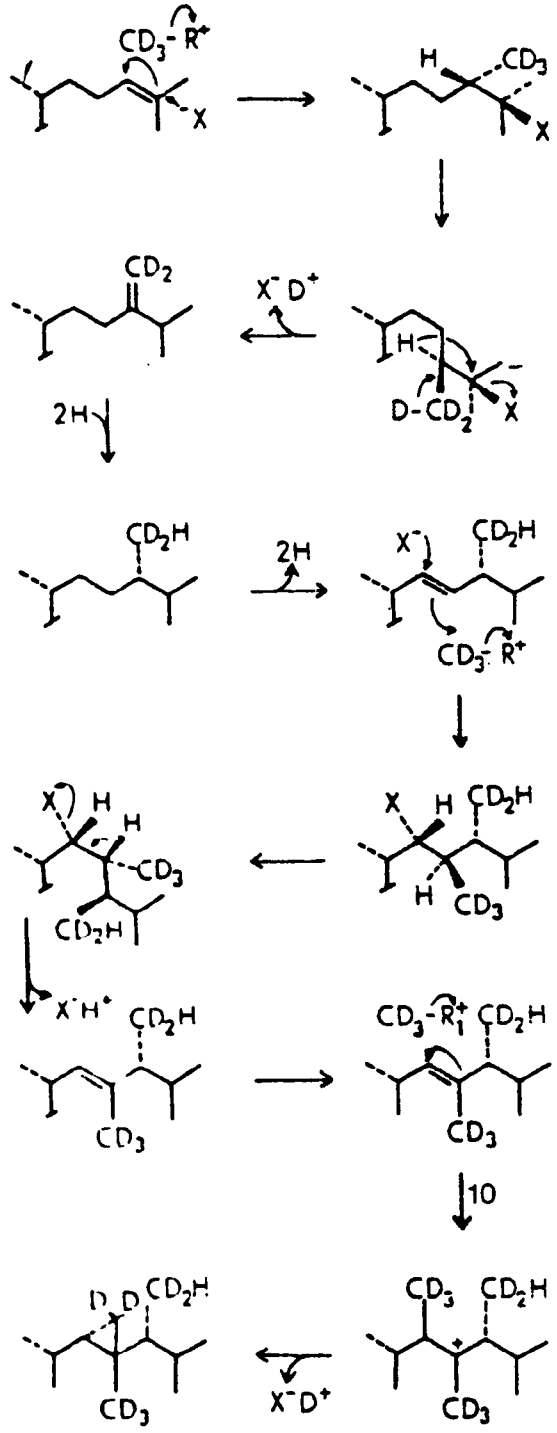


Fig. 3.3/1. Postulated mechanism for biosynthetic side-chain alkylation at C-22, C-23 and C-24 in dinosterol and gorgosterol production from exogenous CD_3-labelled methionine.

(Reproduced from Withers, 1983)

been provided by the detection of 23-methylcholesta-5,22-dien-3β-ol and 23-methyl-5α-cholest-22-en-3β-ol in marine dinoflagellates (Kokke et al., 1979; Kobayashi et al., 1979; Withers et al., 1982; Jones et al., 1983).

A proposed pathway for the biosynthesis of a saturated ring system in dinoflagellates is shown in Fig. 3.3/2. The presence of 4α-methyl-24-methylenecholest-8(14)-en-3β-ol in members of the genus Amphidinium (Withers et al., 1979c; Kokke et al., 1981a) supports the proposed role of this sterol as an intermediate in the above pathway. The two demethylation steps at C-14 and C-4 are based on cholesterol biosynthesis. The pathway proposed by Goad for the saturation of the ring system from a Δ^7-4α-methyl precursor (Withers et al., 1978) is supported by the reported presence of 4α,23,24-trimethycholesta-5,22-dien-3β-ol and dinosterone in dinoflagellates (Withers et al., 1978; Jones et al., 1983; Robinson et al., 1984a).

Of the two proposed pathways for side-chain bioalkylation in dinoflagellates, that shown in Fig. 3.3/1 best accounts for the sterols found in P. lomnickii, P. cinctum, C. hirundinella and W. coronata, i.e. methylation at C-24 prior to methylation at C-23. 4α-Methyl-5α(H)-cholestan-3β-ol could be formed by reduction of the presumed precursor, 4α-methyl-5α(H)- cholest-24-en-3β-ol. Similarly dinostanol could be biosynthesised by reduction of the Δ^{22} double bond in dinosterol, or, alternatively, by quenching of the carbonium ion intermediate produced at step 10 in the postulated biosynthesis of dinosterol (Fig. 3.3/1). Two speculative pathways have been suggested for the formation of 4α,23R,24R-trimethyl-5α(H)-cholest- 17(20)-en-3β-ol: (a) double

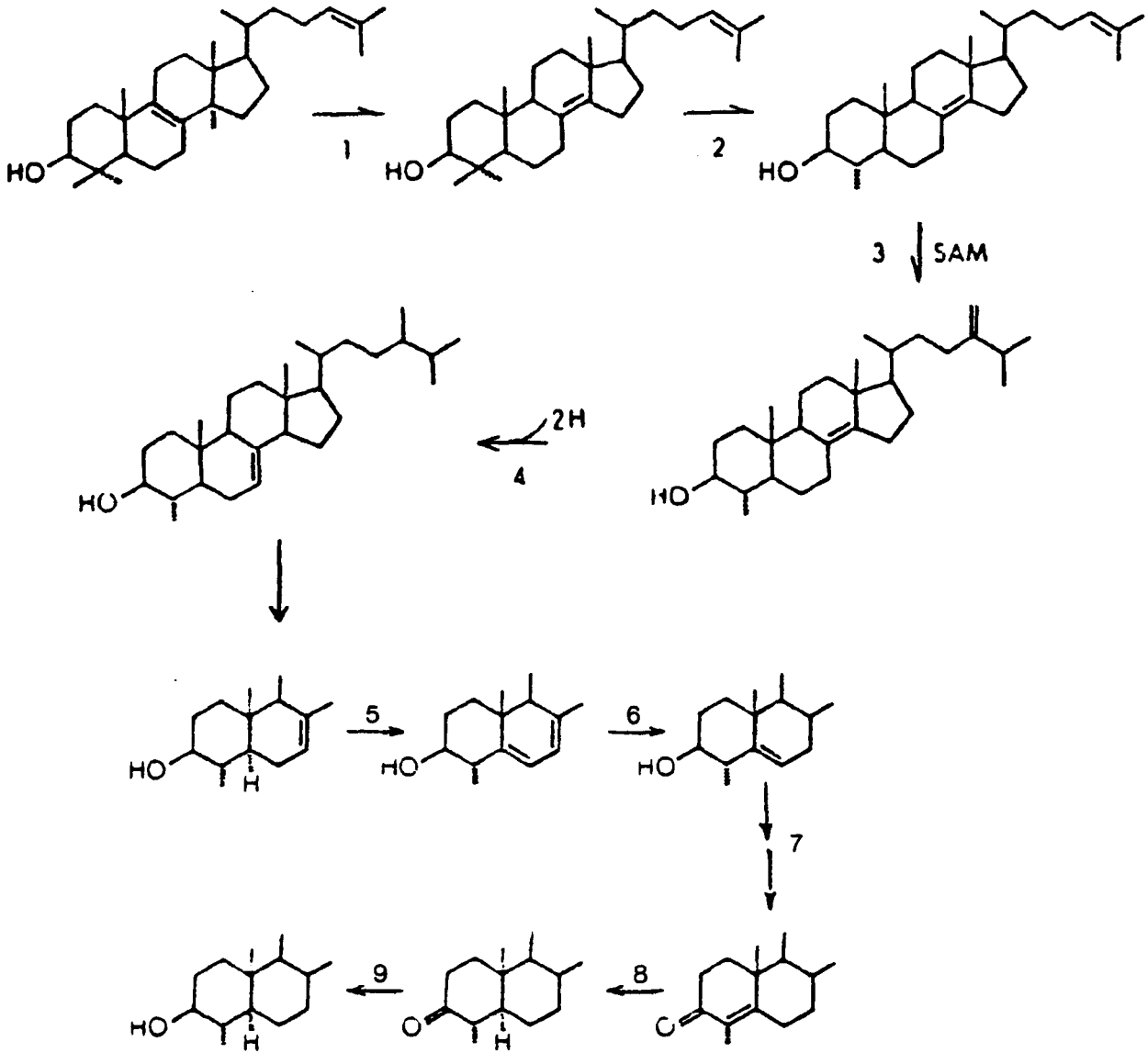


Fig. 3.3/2. Postulated scheme for the partial biosynthetic sequence of dinoflagellate sterols showing amphisterol (XIk) as an intermediate. Steps 1 and 2 are the postulated demethylation steps at C-14 and C-4 respectively. Step 3 indicates side-chain alkylation via SAM (S-adenosylmethionine) followed by hydrogenation of the 24-methylene group and migration of the 8(14) double bond to the 7 position in step 4. Steps 5 and 6 involve formation of a double bond at the 5 position and hydrogenation of the 7 double bond respectively. Steps 7 and 8 show the involvement of ketone intermediates and step 9 reduction of the carbonyl group.
(Adapted from Withers, 1983)

bond migration from the Δ^{22} double bond of dinosterol or (b) isomerisation of a 23-desmethylgorgosterol precursor to a Δ -23,24-dimethyl intermediate followed by double bond migration to the $\Delta^{17(20)}$ isomer (Swenson et al., 1980).

The relative sequence of side-chain bioalkylation and ring system saturation in dinoflagellate sterol biosynthesis is unclear. The presence of 4α,23,24-trimethylcholest-5,22-dien-3β-ol in P. lomnickii, P. cinctum and W. coronata shows that, for these dinoflagellates, the 23,24-dimethyl-Δ^{22} side-chain could be formed before complete saturation of the ring system. 5α(H)-Cholestan-3β-ol, present in P. lomnickii, P. cinctum and C. hirundinella could be formed by demethylation of a 4α-methyl-5α(H)-cholestanol precursor. However, the presence of 5α(H)-cholest-7-en-3β-ol in P. lomnickii suggests that an alternative pathway may be operating, where steps 5-9 of Fig. 3.3/2 take place after loss of the 4α-methyl group, as in the production of 5α(H)-cholestanol in animal tissues (Smith and Goad, 1975). The presence of a high proportion of C_{27} desmethylsterols in P. lomnickii, P. cinctum and C. hirundinella indicates that the previously discussed dichotomy of 4α-methylsterol and desmethylsterol biosynthesis occurs in these species.

Whereas W. coronata does not produce any 4-desmethylsterols, C. hirundinella produces only desmethylsterols. This may be a reflection of their respective evolutionary status, although W. coronata may have possessed the ability to demethylate the 4α-methyl in the past and has since relinquished this ability. In spite of the lack of typical dinoflagellate 4α-methylsterols

in C. hirundinella it contains the desmethyl analogues of many of them. Possibly side-chain bioalkylation precedes 4α-methyl demethylation in this organism, but the presence of 24-methyl-5α(H)-cholest-24(28)-en-3β-ol appears to provide good evidence that this is not necessarily true.

Gorgosterol (22,23-methylene-23,24-dimethylcholest-5-en-3β-ol), which was first isolated from the gorgonian Plexaura flexuosa (Bergmann, 1962), has since been detected in many invertebrates containing zooxanthellae. When the zooxanthellae from various invertebrates were isolated and cultured free from their hosts, it was found that the sterols produced were mainly 4-methylsterols, but that none of the 4-desmethyl-cyclopropylsterols found in the algal/host consortia were present (Kokke et al., 1981b). Also, the levels of 4-desmethylsterols were always much lower than in the algal/invertebrate association. Hence, it was suggested that the dinoflagellates provided dinosterol as a precursor and that the host carried out the final steps in the production of gorgosterol (i.e., demethylation at C-4 and cyclopropanation at C-20 - C-22) (Kokke et al., 1981b). This conclusion was strengthened by the isolation of 4α-methylgorgostanol, gorgosterol and gorgostanol from the dinoflagellates P.foliaceum and P.balticum, which are algal/algal symbiont associations of a heterotrophic dinoflagellate harbouring a photosynthetic haptophyte alga as an endosymbiont (Alam et al., 1979a; Withers et al., 1979a). Finally, an aposymbiotic alga, the dinoflagellate isolated from the Hawaiian anemone Aiptasia pulchella, was found which was capable of synthesising gorgosterol and 23-desmethylgorgosterol in host free

cultures (Withers et al., 1982).

The identification of gorgosterol and gorgostanol in C. hirundinella provides the first report of these cyclopropyl containing sterols in a freshwater alga. Furthermore, C. hirundinella has no known symbiotic existence to enhance the production of these unique dinoflagellate sterols. The presence of 4α-methylgorgostanol in certain dinoflagellates indicates that perhaps cyclopropanation precedes demethylation at C-4 for gorgosterol/gorgostanol formation (Fig. 3.3/3), however, this sequence is as yet unknown and 23,24-dimethylcholest-5,22-dien-3β-ol appears to be the most likely precursor for gorgosterol (Withers et al., 1982). The ring saturated analogue of this precursor was identified in C. hirundinella.

Previously only two algae have been reported to contain dinosterone, both of them marine dinoflagellates (Withers et al., 1978; Jones et al., 1983). The occurrence of a suite of 4α-methylsteroidal ketones in P. lomnickii and W. coronata is unprecedented. The common biosynthetic origin of the 4α-methylsterols and 4α-methylsteroidal ketones in these organisms is demonstrated by their similarity of distribution (Fig. 3.3/4). Alkylation of the side-chain in biosynthesis of the 4α-methylsteroidal ketones may take place before or after formation of the 3-oxo group, although the presence of 4α,23,24-trimethylcholesta-5,22-dien-3β-ol in P. lomnickii and W. coronata suggest that the former is more likely. Cholest-4-en-3-one has been isolated from the marine dinoflagellate Pyrocystis lunula; the presence of this compound in C. hirundinella together with 5α(H)-cholestan-3-one, is

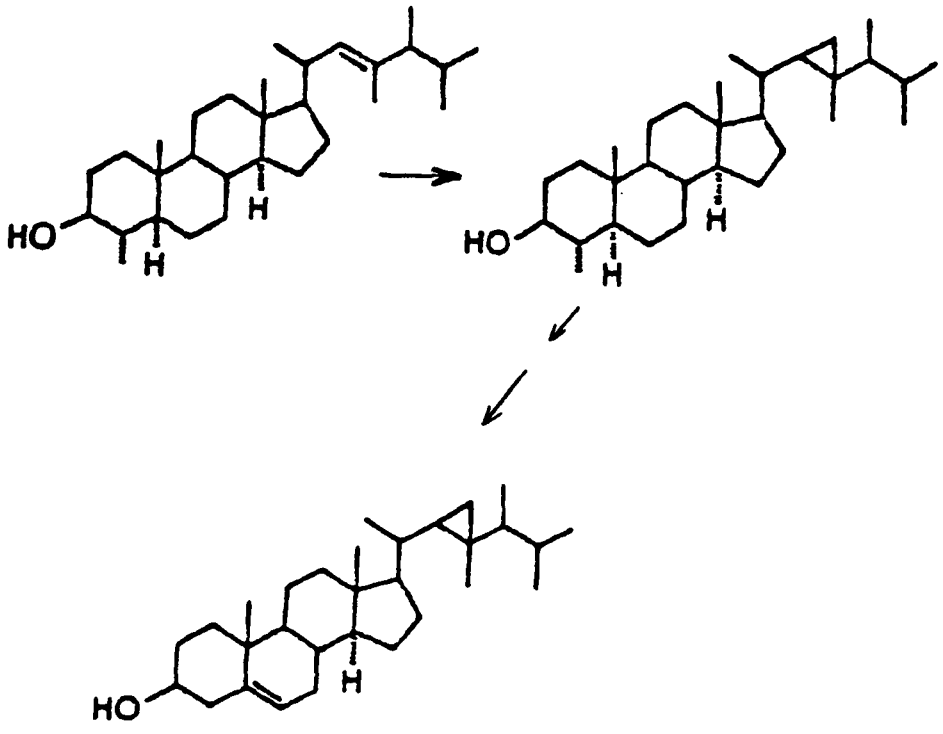


Fig. 3.3/3. Postulated (Djerassi, 1981) biosynthetic conversion of dinosterol to gorgosterol <u>via</u> 4α-methylgorgosterol in dinoflagellates.
(Reproduced from Withers, 1983)

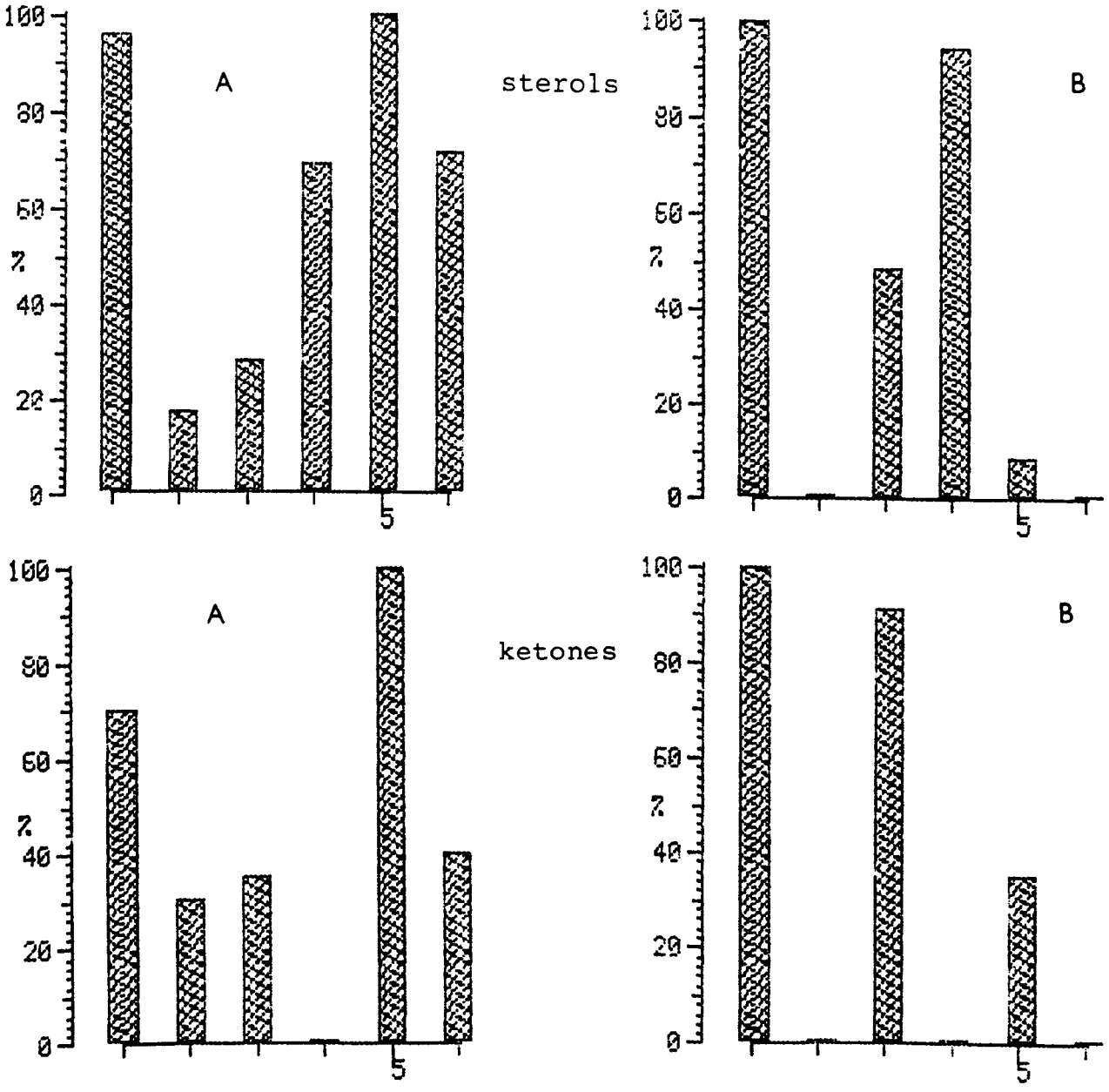


Fig. 3.3/4. Distributions of 4α-methylsterols and 4α-methylsteroidal ketones in: A P. lomnickii and B W. coronata; showing the common biosynthetic origin of these two compound classes.

Key:

1 = 4α-methylcholestan-X 2= 4α,24-dimethylcholest-22-en-X
3= 4α,24-dimethylcholestan-X 4 = 4α,23,24-trimethylcholesta-5,22-dien-X 5 = 4α,23,24-trimethylcholest-22-en-X
6 = 4α,23,24-trimethyl-5α(H)-cholestan-X
X = 3B-ol or 3-one.

consistent with its proposed role in the formation of 5α(H)-cholestanol (Smith and Goad, 1975).

Many of the sterols identified in the four species of freshwater dinoflagellates studied are apparently unique to dinoflagellates; thus, they have the potential to be good marker compounds for dinoflagellate input to sediments. Indeed, most of the sterols identified in these freshwater dinoflagellates have been reported to occur in lacustrine sediments (Cranwell, 1982; Robinson et al., 1984a). The occurrence of relatively high levels of 5α(H)-stanols in P. lomnickii and C. hirundinella has important consequences in organic geochemistry because such compounds are rare in organisms, but are often found in surface sediments (Robinson et al., 1984a). The identification of a series of 4α-methylsteroidal ketones in P. lomnickii and W. coronata demonstrates that such compounds in sediments can arise from direct input by dinoflagellates. The absence of 4α-methylsterols in C. hirundinella and the low level of dinosterol in W. coronata show that care must be taken in the use of dinosterol as a molecular marker for dinoflagellate activity; its absence in sediments may not reflect an absence of dinoflagellates in the phytoplankton. Dinoflagellates have been shown to produce large amounts of sterols relative to cell weight and the 4α-methyl group appears to confer greater resistance to degradation on 4α-methylsterols, thus care must be taken in assessing the importance of a dinoflagellate input to sediments.

From a chemotaxonomic point of view, it is interesting that the two Peridinium species share many sterols in common (Table 3.2/3). The family Woloszynskiaceae is thought to be closely

related to the family Gymnodiniaceae (Bourelly, 1970); such a relationship may be reflected in the lipid composition, 100% of the sterols of W. coronata being 4α-methylsterols whilst a high proportion (93.4%) of the sterols of the marine dinoflagellate Gymnodinium Wilczeki have been reported to be 4α-methylsterols (Nichols et al., 1984). All members of the genus Amphidinium (Family Gymnodiniaceae) which have been analysed for their sterol content synthesise amphisterol (XIk) and no dinosterol (Withers et al., 1979c; Kokke et al., 1981a). At present, however, it would be premature to use sterol compositions as a chemotaxonomic criterion for distinguishing groupings within the Dinophyceae; the vast majority of species have not been analysed and exceptions can exist, for example, Gonyaulax polygramma has a very different sterol composition compared with other members of its genus (Volkman et al., 1984).

Almost all dinoflagellate species that have been examined for steryl ester content have proven to contain such compounds (Withers, 1983). The fatty acid pattern of these steryl esters has been found to be similar to that of the free fatty acid fraction (Withers and Nevenzel, 1977). The sterol composition and distribution of steryl esters can differ from that of the free sterols in the same organism; however, all of the identified steryl esters in the four dinoflagellate species of the present study were composed of sterols present in the free lipid fraction. Steryl esters are presumably metabolic storage forms for sterols in the algal cell (Nes and Mckean, 1977). The presence of steryl esters in marine (De Leeuw et al., 1983) and lacustrine sediments (Cranwell and Volkman, 1981) shows that the

record of algal input to sediments can be preserved in this way.

3.3.ii Other lipids

The hydrocarbons of marine dinoflagellates are particularly rich in heneicosahexaene ($C_{21:6}$) (Blumer *et al.*, 1971). This compound was the dominant constituent of the hydrocarbons of *P. cinctum*, *C. hirundinella* and *W. coronata*, demonstrating that it can occur in freshwater as well as marine environments. Heneicosahexaene has been reported to occur in a surficial intertidal sediment (Thompson and Eglinton, 1978), in which it was postulated to arise from a direct input from photosynthetic diatoms, dinoflagellates and other algae known to contain this alkene. Polyenes, however, have rarely been reported in aquatic sediments and, from its structure, heneicosahexaene would be expected to be rapidly degraded in the aquatic environment under most conditions. 6,10,14-Trimethylpentadecan-2-one has previously been found to occur in *C. hirundinella* (Cranwell, 1976a). Labelling studies have shown that this compound can be formed from phytol (Brooks and Maxwell, 1974), thus the presence of 6,10,14-trimethylpentadecan-2-one in the four species of freshwater dinoflagellates studied presumably reflects chlorophyll diagenesis. This C_{18} isoprenoid ketone occurs widely in sediments.

The molecular composition of the alkyl ester fraction isolated from *P. lomnickii* is presented in Table 3.3/1 together

Table 3.3/1 Molecular composition of wax esters from natural P. lomnickii bloom and from Priest Pot 0 - 6 cm sediment

C no.[a]	Alkyl - acyl[b]		% of chain length[c]	
			P. lomnickii	Priest Pot 0-6 cm
34	18	16	70	91
	20	14	21	2
	16	18	9	7
38	22	16	58	47
	24	14	22	-
	20	18	20	11
42	26	16	77	62
	22	20	13	18
	24	18	10	15
44	26	18	82	80
	28	16	12	9
	22	22	3	6
	24	20	2	5
46	28	18	38	41
	26	20	31	33
	30	16	18	4
	24	22	13	14

(a) Number of carbon atoms.

(b) Molecular composition. First number is the alkyl chain length, second is the acyl chain length.

(c) Different alcohol-acid pairings of the same total carbon number and branching pattern coeluted in GC.
% molecular compositions were determined from the relative proportions of $(RCO_2H)^{\bullet+}$ fragments in the mass spectrum, obtained by summing over the whole peak.

with the % chain length distribution of these compounds in the surface sediment of Priest Pot. The absence in the sediment of certain of the alkyl esters found in P. lomnickii suggest that this dinoflagellate is not a major source of the sedimentary alkyl esters. Similarities in the distribution of the C_{42} and C_{46} alkyl esters from P. lomnickii and the sediment may reflect small amounts of extraneous material contaminating the natural dinoflagellate population. Phytyl esters have been reported to occur in a marine dinoflagellate (Withers and Nevenzel, 1977). In this organism they were esterified to the typical fatty acids found amongst the free lipids of marine dinoflagellates, e.g., $C_{18:5}$. The phytyl esters detected in P. lomnickii and P. cinctum were found esterified to the fatty acids apparently common in freshwater dinoflagellates, e.g., $C_{14:0}$, $C_{16:0}$, $C_{18:1}$.

Triglycerides are commonly found in organisms, where they appear to serve as storage lipids. In the four species of dinoflagellates studied, the acyl units are the same as those used by the organisms for the synthesis of steryl esters. Triglycerides have been detected in particulates collected from sediment traps in oceanic environments (Wakeham, 1982; Wakeham et al., 1983) and some can survive intact to the sediment, having been detected in lacustrine sediments up to 10 000 years old (Dr. P.A.Cranwell, personal communication).

The identification of ethyl and methyl esters in P. cinctum, C. hirundinella and W. coronata, is of interest in view of the sparsity of previous reports of such compounds in organisms. C_{14}-C_{24} ethyl esters have been isolated from the liverwort Conocephalum conicum (Matsuo et al., 1980) and $C_{16:0}$, $C_{18:1}$ and

$C_{18:0}$ ethyl esters have been detected in the phycomycete Rhizopus arrhizus, together with methyl esters (Laseter and Weete, 1971). Interestingly, ethyl esters have been reported to occur in the 50 million year old lacustrine Messel oil shale, which contains abundant levels of dinoflagellate sterols (Habermehl and Hundrieser, 1983; Chapter 6, this thesis).

Marine dinoflagellates commonly contain high levels of polyunsaturated fatty acids (Harrington et al., 1970; Jones et al., 1983; Nichols et al., 1984). The $C_{22:6}$ fatty acid is probably the precursor of heneicosahexaene in dinoflagellates (Blumer et al., 1971). In the four species of freshwater dinoflagellates studied, no fatty acids with more than two double bonds were detected, although C. hirundinella has been reported to contain polyunsaturated fatty acids having up to six degrees of unsaturation (Cranwell, 1976). The sample of C. hirundinella used in the present study was a cultured population, whilst that studied by Cranwell (1976) was a natural population; possibly this may be the cause of the differences found in the fatty acid composition. Although fatty acids were abundant amongst the lipids of C. hirundinella as studied by Cranwell (1976) the total lipids had been hydrolysed. Of the four dinoflagellate species investigated in the present study only P. cinctum contained significant levels of free fatty acids, the majority of the fatty acids in these species were present in an esterified form associated with steryl esters, phytyl esters, alkyl esters, ethyl and methyl esters and triglycerides.

The lipid composition of dinoflagellates can be affected by their growth stage. Thus P. foliaceum contains 3x as much lipid

in the stationary phase, when it is particularly rich in neutral lipids, as in the log phase (Withers and Nevenzel, 1977). Dinoflagellate cysts have not been analysed for their lipid composition, but can occur at very high levels in sediments (Tappan, 1980).

3.4 CONCLUSIONS

The extractable lipid composition of the freshwater dinoflagellates Peridinium lomnickii, P. cinctum, Ceratium hirundinella and Woloszynskia coronata have been determined. Many of the compounds identified have not been reported previously to occur in freshwater dinoflagellates. Freshwater dinoflagellates have been demonstrated to be potential contributors of a wide range of lipids to lacustrine sediments.

1) Freshwater dinoflagellates are capable of synthesising a wide range of 4α-methylsterols and desmethylsterols, in common with marine species. Biosynthesis of sterols in dinoflagellates is complex, a number of different pathways being possible and the particular route taken may differ from one sterol to another. Many of these sterols occur in lacustrine sediments, in which they probably reflect a dinoflagellate input.

2) 4α-Methylcholestan-3-one, 4α,24-dimethylcholest-22-en-3-one, 4α,24-dimethylcholestan-3-one and 4α,23,24-trimethylcholestan-3-one have been isolated from organisms for the first time, together with dinosterone, previously reported to occur in

marine dinoflagellates. Sedimentary 4α-methylsteroidal ketones may, therefore, arise from a direct input by dinoflagellates.

3) Steryl esters occur in each of the four species of dinoflagellates studied. A similar range and distribution of sterols were present in an esterified form as present in a free form in individual species. Steryl esters provide another form in which steroids may be deposited into sediments.

4) Methyl and ethyl esters occur in P. cinctum, C. hirundinella and W. coronata, providing the first report of ethyl esters in a major group of algae.

5) Phytol can occur esterified to fatty alcohols in freshwater dinoflagellates.

6) Heneicosahexaene occurs as the dominant hydrocarbon in P. cinctum, C. hirundinella and W. coronata, as in many marine dinoflagellate species, although the presumed precursor $C_{21:6}$ fatty acid was not detected.

7) Fatty acids were largely present in an esterified form.

8) Triglycerides are apparently acting as an important energy store for the organisms.

Profitable work for the future would be the investigation of changes in lipid composition of dinoflagellates at different growth stages and the determination of the lipid composition of dinoflagellate cysts.

CHAPTER FOUR

PRIEST POT

4.1 INTRODUCTION

Studies of the fate of lipids in present day sedimentary systems may be used in the elucidation of the depositional environments of ancient sediments from their geolipid distributions. Many workers have analysed various source inputs to the sediments to try to find lipid distributions characteristic of restricted groups of organisms for correlating with geolipid assemblages, or, in the extreme case, to find a biolipid unique to one species of organism, which gives rise to a geolipid that may be used as a biological marker compound for input from that organism. Modifications of these lipids commence within the water column and continue as diagenetic changes after incorporation into the sediments. These changes may be brought about by chemical or biological mechanisms, with the most intense degradation occurring at the sediment/water interface where bacterial concentrations are greatest.

Methods for investigating changes in lipid distribution within the water column include (a) simplified laboratory studies of the marine food web, in which the lipid content of zooplankton faecal pellets is compared with that of phytoplankton, the food source (e.g. Eglinton et al., 1979) and (b) the deployment of sediment traps at various depths in the open ocean (e.g. Wakeham et al.,1980c). We have utilised a different approach to study this problem in Priest Pot, a small eutrophic lake in the English Lake District (Fig. 4.1/1).

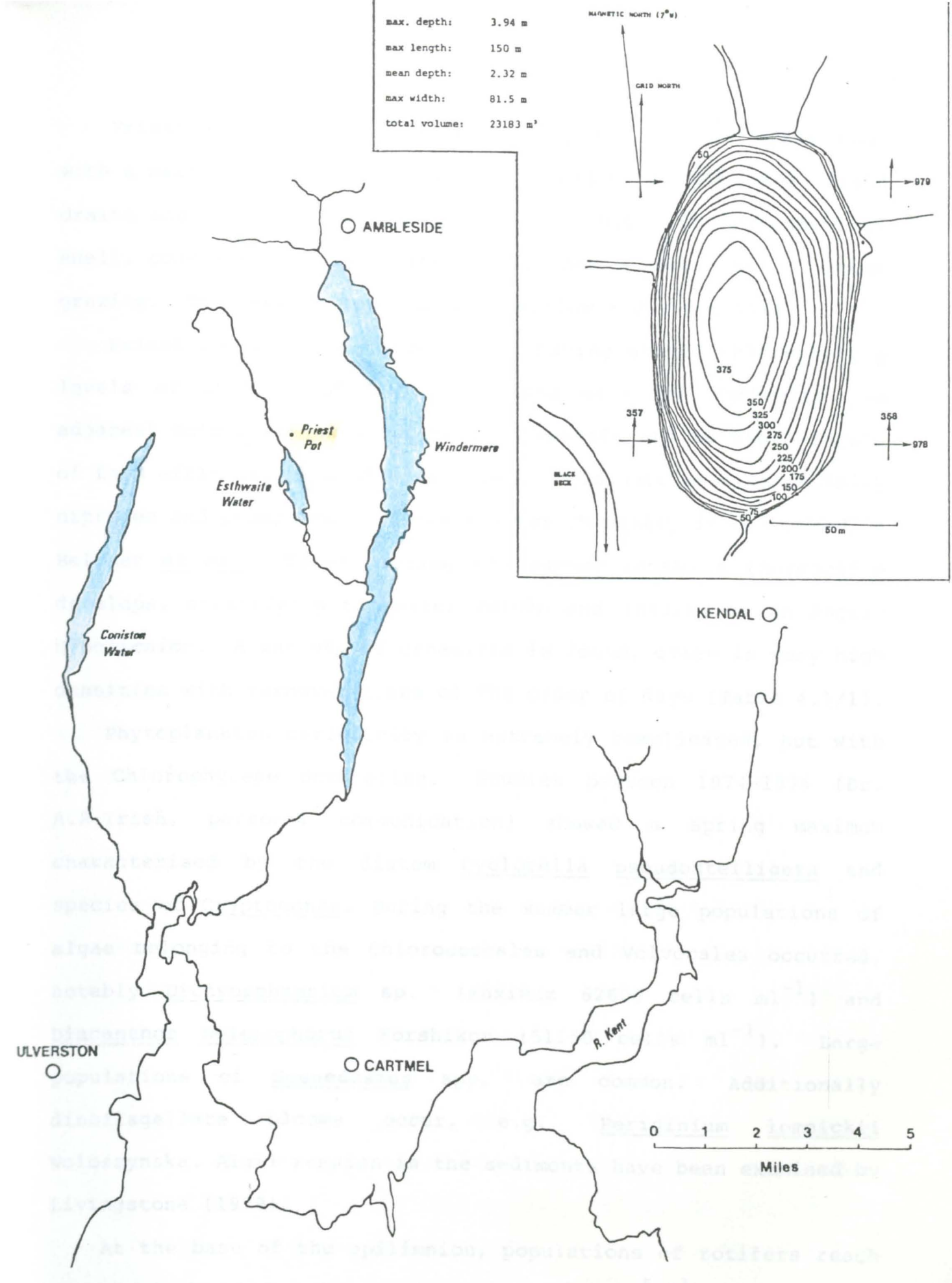


Fig. 4.1/1. A sketch map showing the location of Priest Pot, Cumbria, England.
Inset: bathymetric contours of Priest Pot.

Priest Pot(54° 22′N, 3° 00′W) is a small ($10^4 m^2$) water body with a maximum depth of 3.94 m. It is fed by ground water, field drains and two or three slow-flowing ditches. The catchment is small, consisting of fertilised fields and a small area of rough grazing. The lake is surrounded by willow and alder carr.

Priest Pot is highly productive, having summer chlorophyll a levels of up to 2300 ug/l (cf. <400 ug/l for Esthwaite, an adjacent eutrophic lake) due to land run-off and possible inputs of farm effluent, both of which are rich in nutrients, especially nitrogen and phosphorus. Surface water chemistry is described in Belcher et al. (1966). During the summer months a thermocline develops, stratifying the water column and leading to an anoxic hypolimnion. A variety of organisms is found, often in very high densities with turnover times of the order of days (Table 4.1/1).

Phytoplankton periodicity is extremely complicated, but with the Chlorophyceae dominating. Studies between 1974-1976 (Dr. A.E.Irish, personal communication) showed a spring maximum characterised by the diatom Cyclotella pseudostelligera and species of Cryptomonas. During the summer large populations of algae belonging to the Chlorococcales and Volvocales occurred, notably Dictyosphaerium sp. (maximum 62600 cells ml^{-1}) and Diacanthos belenophorus Korshikov (51150 cells ml^{-1}). Large populations of Scenedesmus spp. are common. Additionally dinoflagellate blooms occur, e.g. Peridinium lomnickii Woloszynska. Algal remains in the sediments have been examined by Livingstone (1979).

At the base of the epilimnion, populations of rotifers reach very high densities in summer (up to $3 \times 10^5 l^{-1}$), grazing the

phytoplankton. Near to the thermocline, but in the anoxic water, communities of large (length generally >100 um) ciliated protozoa develop in summer (Finlay et al., 1983). These ciliates are dominated by two species of the primitive genus Loxodes and often reach extremely high densities (up to $8 \times 10^5 l^{-1}$). A community of anoxygenic sulphide-oxidising photosynthetic bacteria, mainly members of the Chlorobiaceae, reach a maximum concentration close to the sediment/water interface (Davison and Finlay, 1984).

Organisms in the water column of Priest Pot occupy regions that fulfill their ecological requirements, with phytoplankton in the surface layers where light intensity is greatest and rotifers, obligate aerobes, occupying the middle to lower epilimnion. Ciliated protozoa, mainly Loxodes magnus and striatus, are eukaryotes, but have been found to contain a dissimilatory nitrate reductase enzyme, allowing them to exist in the anoxic zone (Finlay et al., 1983), thus avoiding competition and filling an ecological niche. Light levels at longer wavelengths are still high enough near to the sediment/water interface to allow photosynthesis by photosynthetic bacteria, the dominant organism of which has been identified as Clathrochloris hypolimnica (Davison and Finlay, 1984). Summer stratification of the water column, therefore, results in vertical stratification of organisms (Fig. 4.1/2) into zones, each of low species diversity and high biological density. Natural populations of rotifers, ciliated protozoa and Clathrochloris hypolimnica bacteria (Table 4.1/1) were obtained from Priest Pot, in order to determine the lipids of these organisms and to elucidate some of the processes operating on lipids between production by the

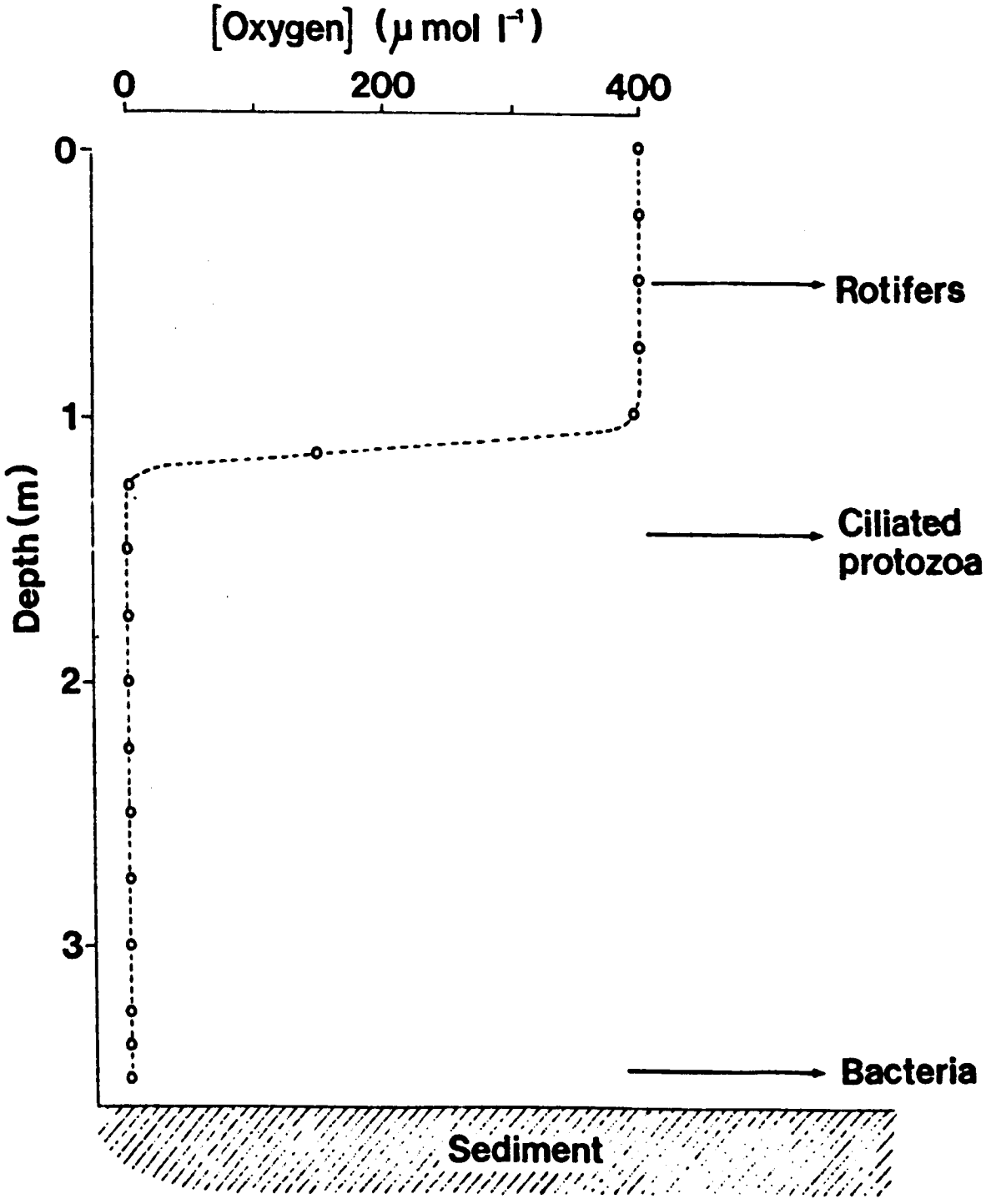


Fig. 4.1/2. Vertical distribution of dissolved oxygen in the water column of Priest Pot on 7 July 1982. Dissolved oxygen was determined using a YSI meter and stirrer-assisted electrode. Three 5 l water samples containing different populations were collected from the indicated positions in the water column using a peristaltic pump.
(Reproduced from Robinson et al., 1984b)

Table 4.1/1 Identity, composition and ecology of organisms from Priest Pot

Depth (m)	Sample	Main species present	Comments
0.5	Rotifers	Keratella cochlearis, K. quadrata, Polyarthra spp. mainly vulgaris; Anuraeopsis fissa	Feeding on bacteria, algae, fine detritus, other rotifers. Short generation time. Predators: other rotifers, fish, some copepods. They do not produce discrete faecal pellets.
1.5	Ciliated protozoa	Loxodes magnus; L. striatus; Spirostomum spp.; Prorodon sp.; Frontonia sp.	Loxodes spp. are 70-80% of total. feed on algae, fine detritus, some rotifers including rotifer eggs. Few predators - excluded by anoxia. Turn-over of organisms is rapid - presumably dead ciliates are sedimenting out, but they are fragile and remains are not visible in the sediment.
3.8	Bacteria	Mainly Chlorobiaceae, but some purple photosynthetic bacteria	Maximum just above sediment/water interface during periods of anoxia. Photosynthetic sulphide-oxidizing bacteria.

primary producers in the euphotic zone and incorporation into the sediments. Although lipids were not determined on axenic cultures, by looking at natural populations the problems of changing the lipid pattern with changes in culture conditions has been avoided. Previously little or no information was available for the lipid constituents of such organisms, which are potential sources of lipids for the sediments of many water bodies. The fatty acids of cultured Chlorobium, a member of the Chlorobiaceae, were recently reported by Knudsen et al. (1982); the only ciliated protozoon studied for its lipid content is Tetrahymena pyriformis (Mallory et al., 1963; Wheeler and Holmlund, 1975) and the sterols of one marine rotifer species have been reported (Teshima et al., 1979).

In order to extend the investigation of lipid transformations in Priest Pot to include those that take place in the sediment during the early stages of burial, a short core of bottom sediment was obtained. The core was sectioned and selected horizons were dated from their Pb^{210} content (see following section). A section from near the bottom of the core, dated to ca. 1900-1910, was analysed for its free and bound lipid composition. Thus, changes in sedimentary lipid composition due to changes in input and/or the effects of lipid diagenesis over the past seventy years, could be investigated for Priest Pot by comparing the lipid distributions of the deeper section with those of the surficial sediments. Evidence from aerial photographs and old picture-postcards, indicates that the present tree cover closely surrounding the lake has developed over the last century, and that at the time the 51-59 cm sediment section

was deposited, tree cover would have been markedly less than at the present. There is no direct evidence concerning the algal productivity at the time the sediment was deposited.

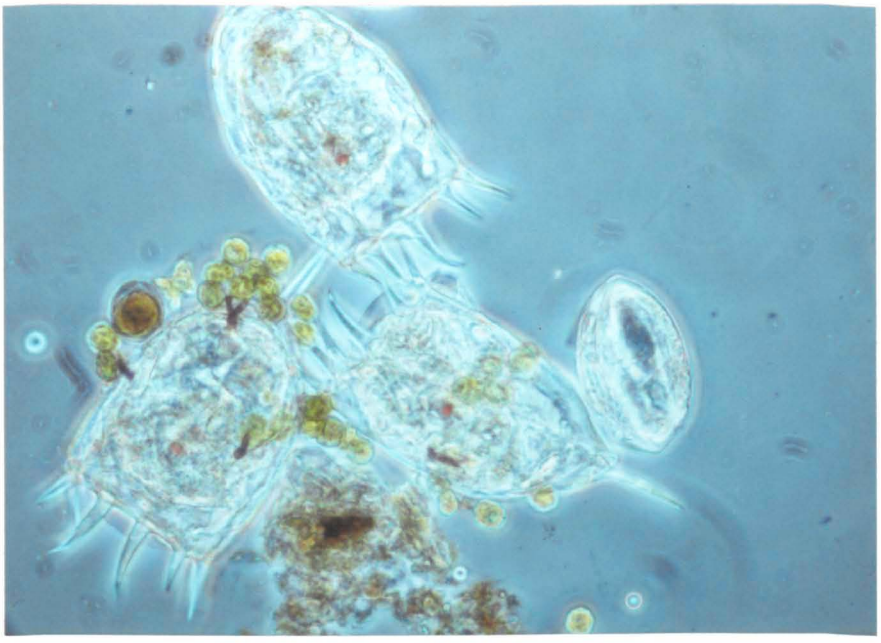
4.2 RESULTS

4.2.i Description of samples

Natural populations of rotifers, ciliated protozoa and photosynthetic bacteria were collected in July 1982 with the assistance of Dr.B.J.Finlay and Mr.C.Woof (Freshwater Biological Association, U.K.) during a period in which stratification was well established (Fig. 4.1/2). Visibility, as determined by Secchi disc, was 50 cm. Samples, collected by plastic tubing connected to a peristaltic pump, were removed from discrete depth levels in which the respective organisms achieved their highest abundances. Microscopic examination (Fig. 4.2/1) indicated that all three samples were >90% pure on a biomass basis (Table 4.1/1).

Surface sediment (0-6cm) was collected by a Mackereth 1 m corer (Mackereth, 1969) in August 1981 during the stratification period. The surface sediments consisted of a black, anoxic, flocculent mud, having a high organic carbon content and few recognisable algal fragments. The bottom water pH was 6.5. A second core was obtained in July 1983 during the stratification

(a) Keratella cochlearis (length 150 um) with egg.

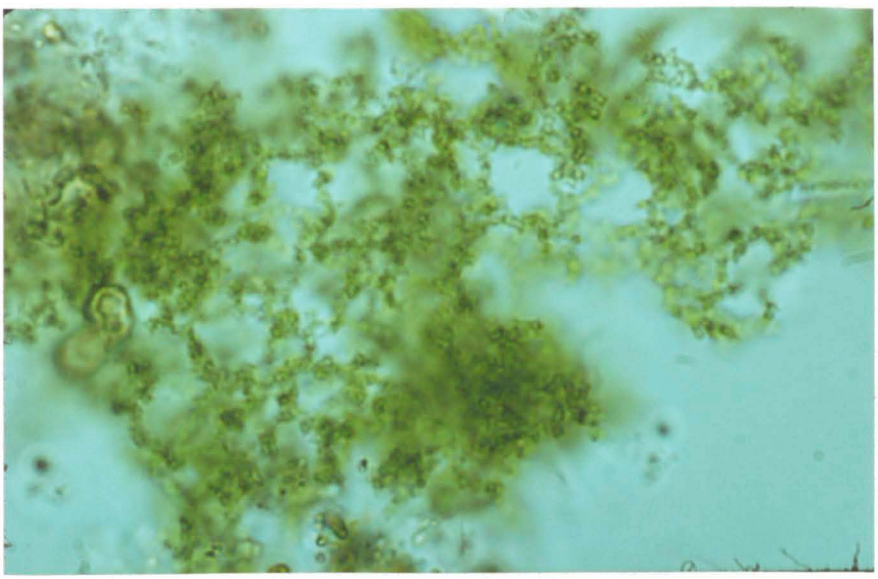


0 150μm

(b) CILIATED PROTOZOA showing: Frontonia leucas, Loxodes striatus, L. magnus and Spirostomum sp.

0 400μm

(c) Clathrochloris hypolimnica.



0 50μm

Fig. 4.2/1. Natural populations of organisms isolated from Priest Pot. A description of the organisms is given in Table 4.1/1.
(a) Rotifers.
(b) Ciliated protozoa.
(c) Photosynthetic bacteria.

period. The stratigraphy of the core was as follows:

0-22 cm black

22-43 cm brown

43-76 cm lighter brown

After compression and gas expulsion the core was 69 cm in length. The water above the surface was green containing abundant C. hypolimnica; in the sediment, the pH of the interstitial water changed gradually from 6.65 at the surface to 6.25 at 61-66 cm. Selected horizons were dated from their Pb^{210} content (Fig. 4.2/2). CHN data for the two cores is given in Table 4.2/1.

Table 4.2/1 CHN composition of Priest Pot 0-6 cm and 51-59 cm sediment sections.

Section[a]	Total[b] %			Organic[b] %		
	C	H	N	C	H	N
0-6 cm	21.6,22.4	2.3,2.9	2.0,2.1	20.1,20.8	2.3,2.3	1.0,1.0
51-59 cm	16.1,16.1	2.1,2.1	0.9,0.9	16.1,16.1	2.1,2.1	0.9,0.9

a) Taken from different cores, see text.
b) Values given are for duplicate measurements.

The abundances of lipids by compound class are presented in Table 4.2/2.

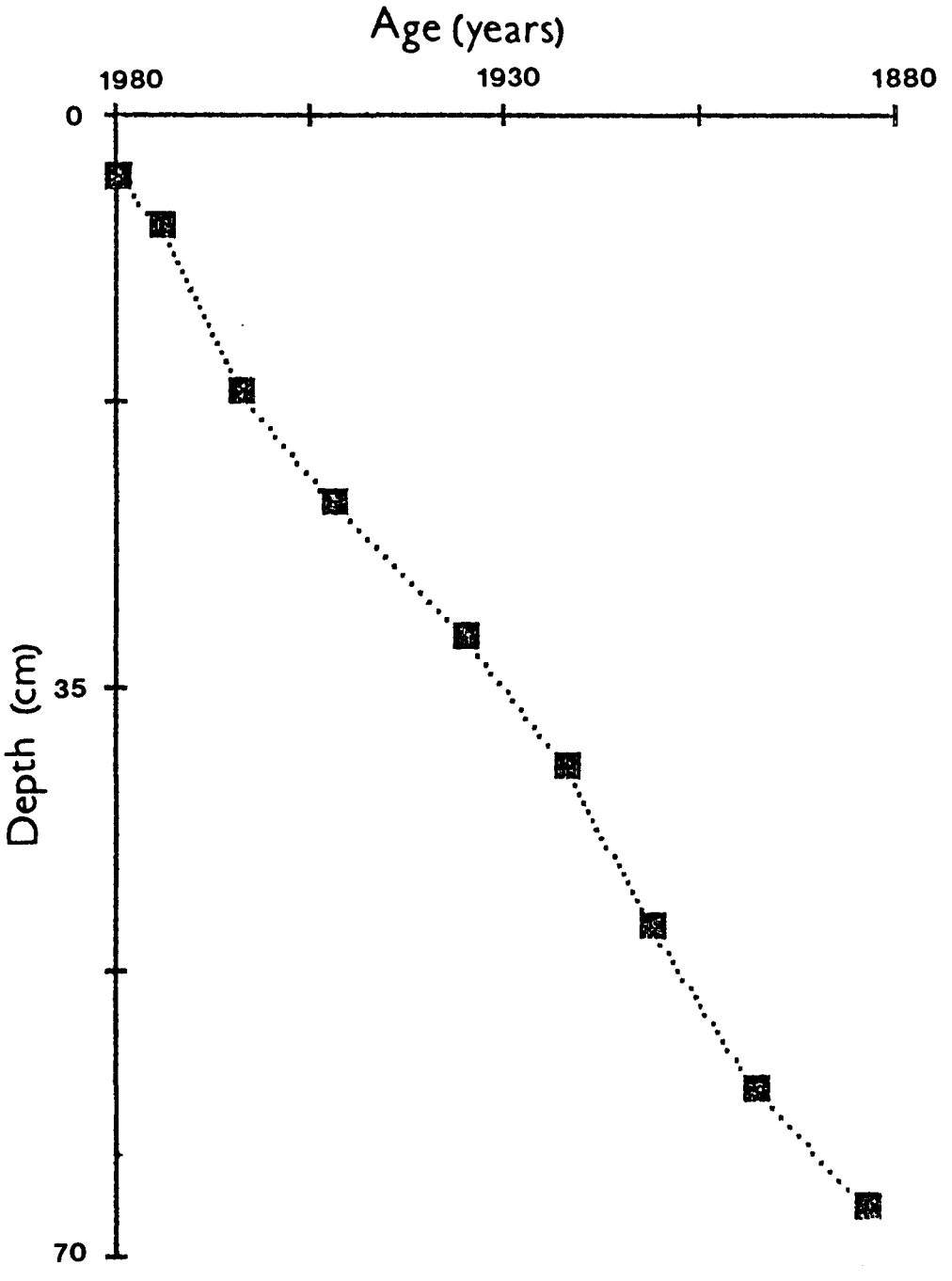


Fig. 4.2/2. Pb^{210} Dating of Priest Pot sediment core collected in 1983 (ages calculated using a crs model). (Analysis performed by Atomic Energy Research Establishment, Harwell)

Table 4.2/2 Compound class abundance in Priest Pot samples

Samples		Hydro-carbons	Acids	Hydroxy Acids	Alkanols	Desmethyl sterols	4-Methyl sterols	Ketones	Aldehydes	Wax Esters
Rotifers[a]	(Total)	1.4	6.4	-	6.2	4.4	0.8	-	-	N.A.
Ciliated protozoa[a]	(Total)	17.0	116	-	10.0	5.1	0.2	-	-	N.A.
Clathrochloris[a]	(Free)	10.5	127	1.4	88.0	74	32.0	3.4	-	-
0 - 6 cm Sediment[b]	(Free)	25.0	10.4	0.5	250	203	111	8.3	3.7	10.6
	(Bound)	0.5	44.0	14.0	4.5	10.1	1.3	-	-	N.A.
51 - 59 cm Sediment[b]	(Free)	23.0	0.6	0.5	220	88	117	7.3	-	*
	(Bound)	17.5	65.0	28.4	81.0	8.3	1.8	5.1	-	N.A.

(-) Not detected.

(N.A.) Not applicable - extraction involved saponification.

(*) Not determined.

(a) Expressed as ug l water^{-1}, obtained by summing abundances of individual components.

(b) Expressed as ug g dry, extracted sediment^{-1}, obtained as above.

4.2.ii Hydrocarbons

The hydrocarbon fraction of the rotifer lipids contained relatively low levels of n-alkanes having a unimodal, smooth distribution maximising at C_{24}. n-Alkanes of both ciliates and C. hypolimnica were dominated by n-C_{17}, with the C. hypolimnica n-alkane distribution also containing a much smaller maximum at C_{29}. The free and bound sedimentary n-alkane distributions are shown in Fig. 4.2/3. Pristane and phytane were identified in the 0-6 cm section (740 and 1200 ng/g respectively), whereas 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane (670ng/g) was isolated from the 51-59 cm section. Isobotryococcene (XLIX), identified from its mass spectrum and relative retention time and those of the product produced upon hydrogenation, was present at an abundance ca. 170% that of n-C_{17} alkane amongst the lipids of the ciliates. Squalene (XLVIII) was present amongst the lipids of C. hypolimnica and the sediments. The free hydrocarbons of the two sediment sections contained a series of alk-1-enes (C_{19}-C_{27}) maximising at C_{25} and showing a marked CPI (Fig. 4.2/4). Also present in the free lipids of the 0-6 cm sediment were three $C_{30:6}$ alkenes, which, from their mass spectra, are probably isomers of squalene, a $C_{30:4}$ alkene tentatively identified as a partly reduced form of squalene and two $C_{20:4}$ alkenes of unknown structure. Phytenes and phytadienes were identified in the free and bound lipids of both sediment sections, with phytadienes being relatively more abundant in the deeper sample.

Steroidal hydrocarbons were detected amongst the lipids of the C. hypolimnica sample and amongst the free lipids of the two sediment sections (Table 4.2/3). Hopanoid hydrocarbons were

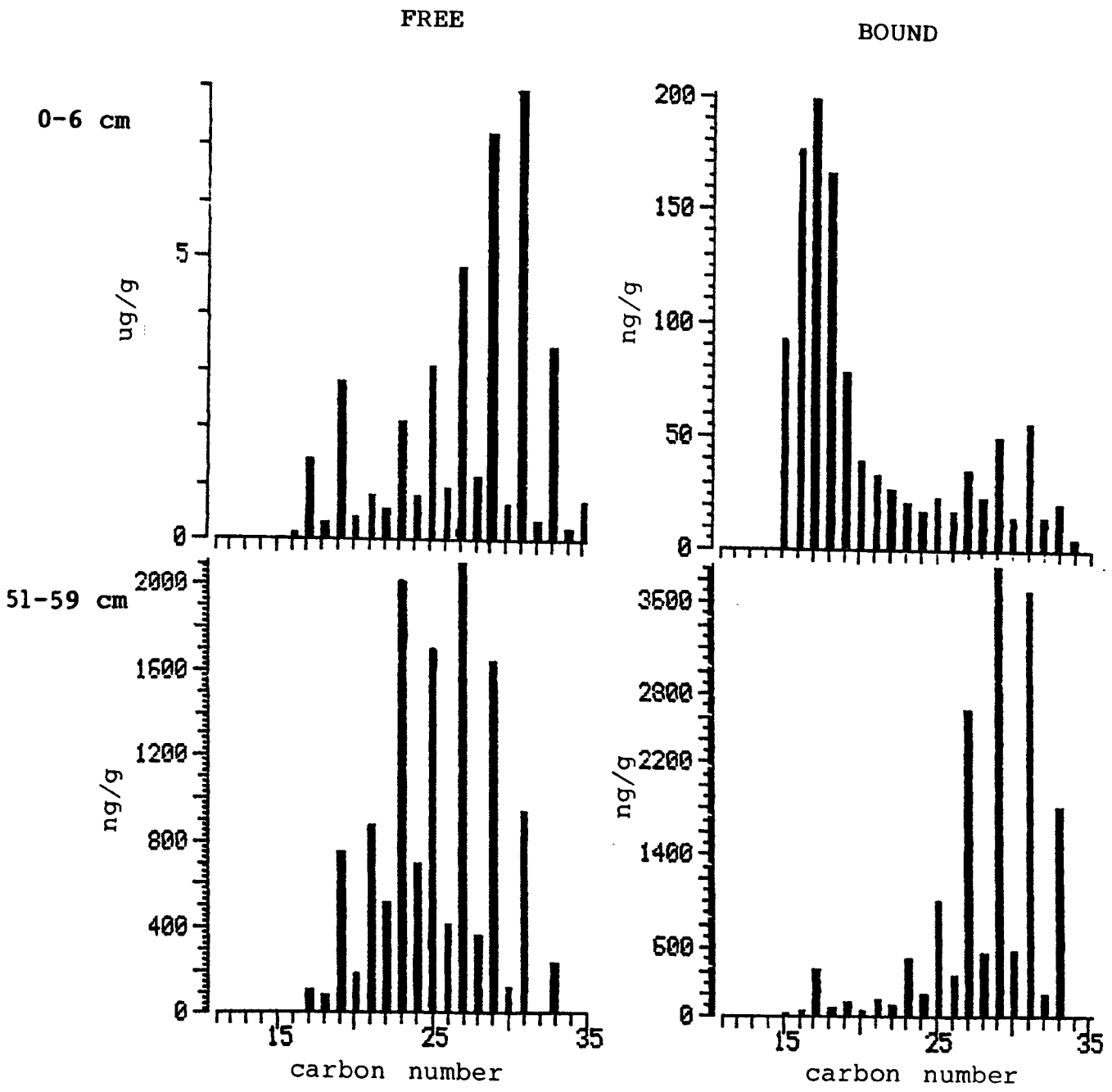


Fig. 4.2/3. Abundances of free and bound n-alkanes in Priest Pot 0-6 cm and 51-59 cm sediment sections. Quantitation expressed as ng (or ug) g dry, extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of n-C_{18} and n-C_{28} alkanes.

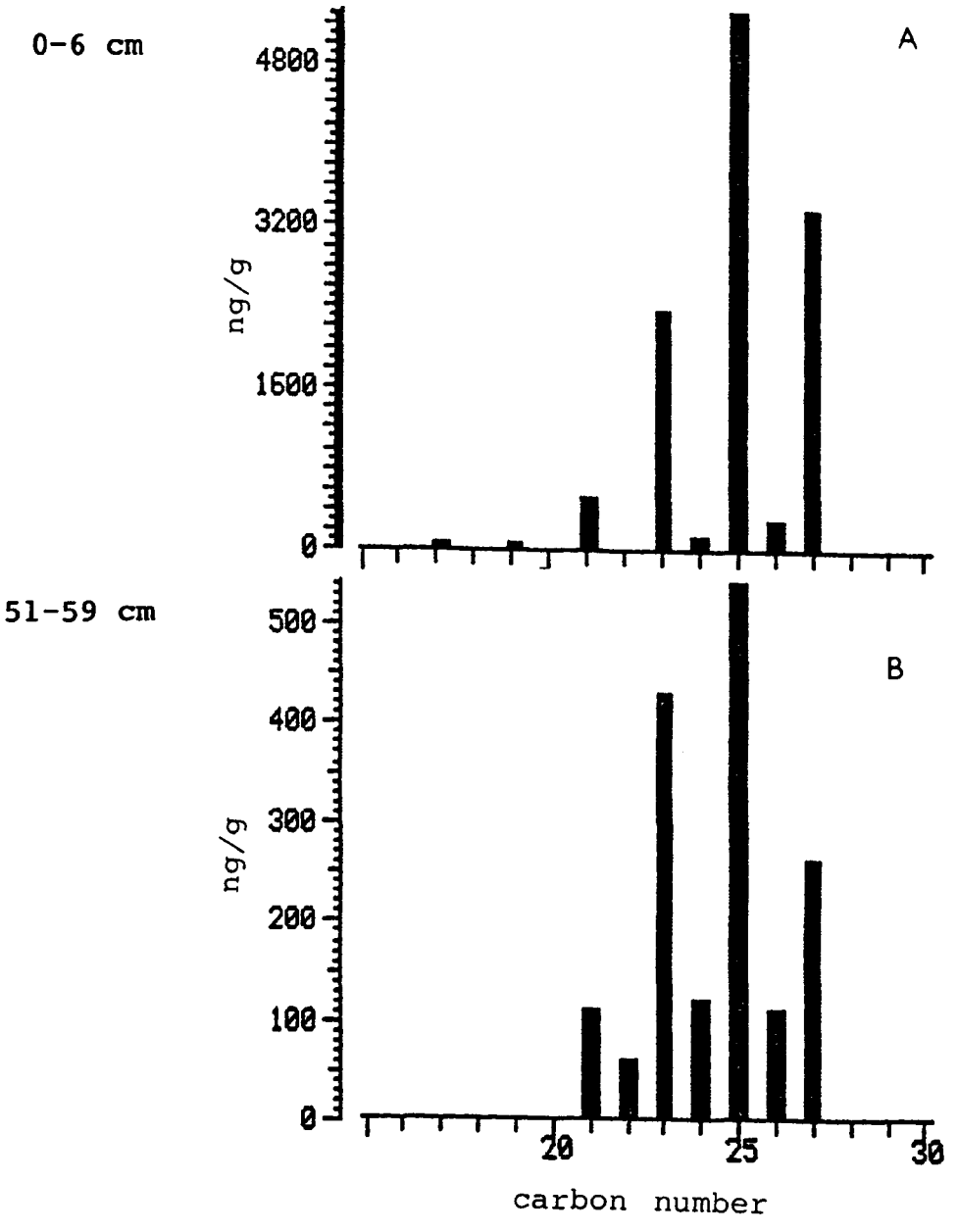


Fig. 4.2/4. Abundances of free alk-1-enes in Priest Pot 0-6 cm and 51-59 cm sediment sections.
Quantitation expressed as ng g dry, extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of n-C_{18} and n-C_{28} alkanes.

Table 4.2/3 Free steroidal hydrocarbon abundances in Priest Pot sediments and *Clathrochloris hypolimnica*

Compound	Structure	*Clathrochloris* (ng l water^{-1})(a)	0 - 6 cm (ng g^{-1})(b)	51 - 59 cm (ng g^{-1})(b)
Cholest-2-ene	XIX a	80	630	100
Cholesta-3,5-diene	XX a	N.D.	260	N.D.
C_{28} triene		N.D.	N.D.	140
24-Methylcholest-2-ene	XIX f	N.D.	130	200
24-Methylcholesta-3,5-diene	XX f	N.D.	340	N.D.
C_{29} triene		N.D.	N.D.	100
24-Ethylcholest-2-ene	XIX h	tr.	490	230
24-Ethylcholesta-3,5-diene	XX h	N.D.	360	180

(a) Abundances expressed as ng l water^{-1}, determined by comparison of GC peak areas with that of a known amount of n-C_{28} alkane.

(b) Abundances expressed as ng g dry, extracted sediment^{-1}, determined as above.

identified amongst the free lipids of both sediment sections (Table 4.2/4). The lipids of the C. hypolimnica sample contained relatively low amounts of hopanoid hydrocarbons, which had a distribution similar to those isolated from the 0-6 cm sediment. Low levels (ca. 100 ng/g) of fern-8-ene and fern-9(11)-ene were detected amongst the free hydrocarbons of the 51-59 cm section. Five compounds were tentatively identified as A ring degraded triterpenoidal hydrocarbons amongst the free lipids of the 51-59 cm sediment section, a sixth was identified as des-A-lupane (100 ng/g) by comparison of its mass spectrum with that of a standard (Hoffmann, 1984).

Aromatic hydrocarbons were present amongst the free lipids of the sediment samples, but were not fully analysed. The deeper sediment sample appeared to contain a greater relative abundance of aromatics; perylene (LXI) was present in the 0-6 cm sediment (1090 ng/g).

The bound lipid fraction largely containing hydrocarbons isolated from the 51-59 cm sediment section, also contained cholesteryl chloride, 24-methylcholesteryl chloride, 24-ethylcholesta-5,22-dienyl chloride and 24-ethylcholesteryl chloride. 24-Ethylcholesteryl chloride was identified by comparison of its mass spectrum with that of a synthesised standard (Fig. 4.2/5). The same fraction also contained C_{27}-C_{29} $\Delta^{3,5}$ steradienes, C_{28} and C_{29} $\Delta^{3,5,22}$ steratrienes, 4-methyl,24-ethyl-19-norcholesta-1,3,5(10)-triene and a C_{29} 14α(H) B ring anthrasteroid. These compounds, however, are thought to be artifacts of the extraction procedure (see Discussion).

Table 4.2/4 Abundances of hopanoid hydrocarbons in Priest Pot sediments

Compound[a],[b]	Structure	Quantitation[c] (ng/g)	
		0 - 6 cm	51 - 59 cm
22,29,30-Trisnorhop-17(21)-ene		-	160
C_{27} Hopene		-	50
17β(H),21β(H)-22,29,30-Trisnorhopane		-	140
30-Norhop-17(21)-ene		90	270
Hop-17(21)-ene	XXXI	60	130
17β(H),21α(H)-30-Norhopane		-	130
17α(H),21β(H)-Hopane	XXXII	130	80
Neohop-13(18)-ene		-	tr.
C_{30} Hopene		30	-
17β(H),21β(H)-30-Norhopane		-	320
17β(H),21α(H)-Hopane	XXXIII	20	tr.
22S-17α(H),21β(H)-Homohopane		10	tr.
22R-17α(H),21β(H)-Homohopane		30	500
17β(H),21β(H)-Hopane	XXXIV	-	70
17β(H),21α(H)-Homohopane		-	tr.
Hop-22(29)-ene	XXXV	2 200	1 300
Hop-21-ene		220	570
22S-17α(H),21β(H)-Bishomohopane		tr.	-
22R-17α(H),21β(H)-Bishomohopane		tr.	tr.
Homohop-29(31)-ene	XXXVI	-	100
17β(H),21β(H)-Homohopane		130	300
17β(H),21β(H)-Bishomohopane		tr.	50

- Not detected; tr. Trace levels present

(a) Compounds identified by comparison of mass spectra and relative retention times with published data (e.g. Barnes et al., 1979; Wardroper 1979).

(b) Traces of C_{33} - C_{35} hopanes were also detected by mass fragmentography.

(c) Quantitation expressed as ng g dry extracted sediment^{-1} and determined by comparison of GC peak areas with that of a known amount of n-C_{28} alkane.

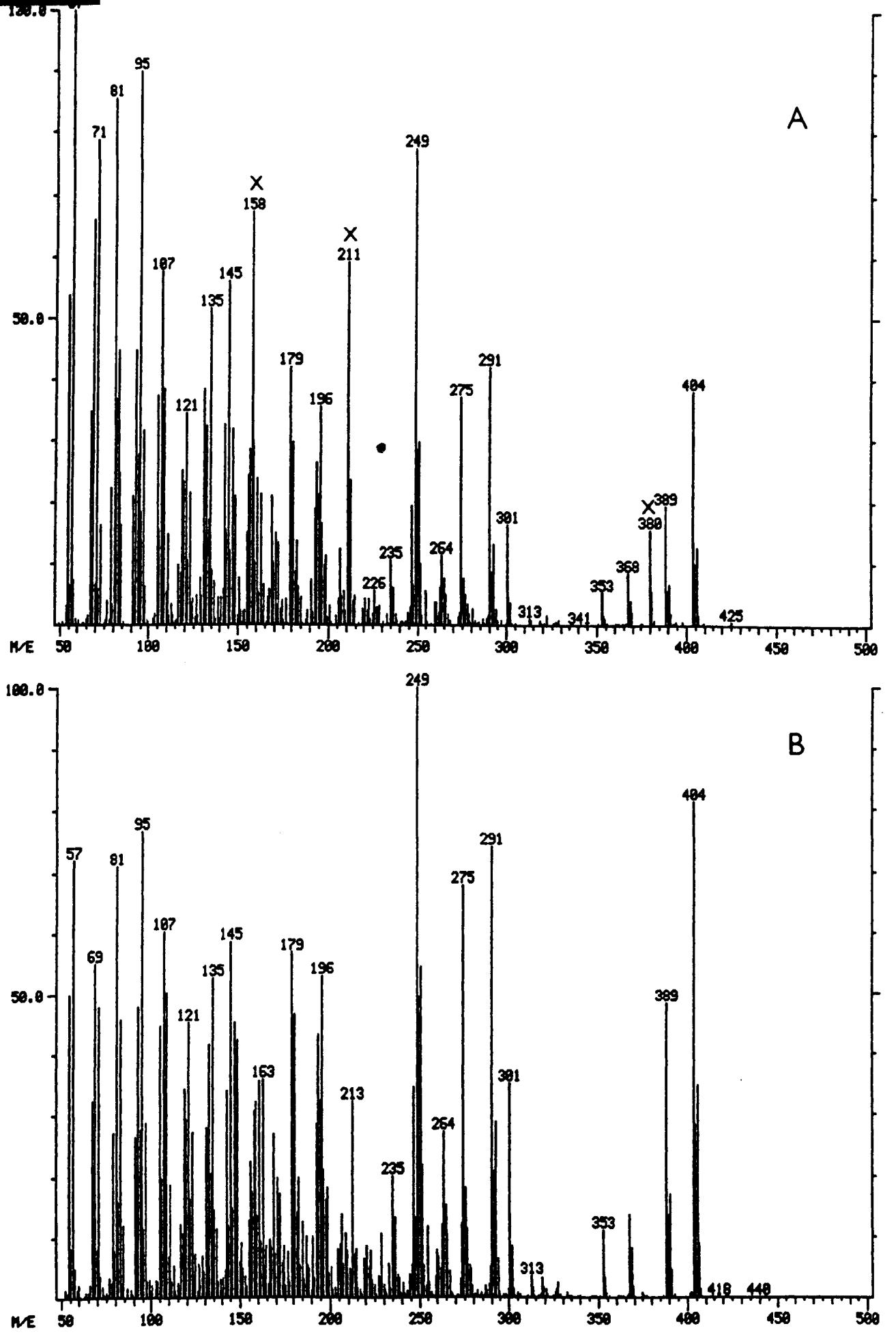


Fig. 4.2/5. Mass spectra of: (A) cholesteryl chloride from the 51-59 cm bound hydrocarbons; (B) synthesised cholesteryl chloride. Ions marked with a X in (A) are due to coelution of a C_{28} 4-methyl A ring monoaromatic steroid. Operating conditions are given in Chapter 8.

4.2.iii Alcohols

a) Acyclic alcohols

Straight chain alcohols between C_{10}-C_{32} were detected; their distributions are shown in Figs. 4.2/6 and 4.2/7. Branched chain alkanols, dominated by iso- and anteiso-C_{15}, were relatively more abundant amongst the bound lipids of the sediment than the free. Phytol was a major component of each lipid fraction except for the bound lipids of the 51-59 cm sediment section where it was absent, although a trace amount of dihydrophytol was detected. Hexadecan-2-ol was detected in the lipids of each organism and the 0-6 cm sample, farnesol (I) was a constituent of the C. hypolimnica sample and the 0-6 cm free lipids (Fig. 4.2/6). A relatively abundant compound (815 ng/g) was observed eluting between n-C_{19} and n-C_{20} alkanols (TMS) having a mass spectrum identical to that shown in Fig. 3.2/7. A series (C_{16}-C_{31}, max. C_{27}) of alkan-2-ols was identified amongst the free alcohols of the 51-59 cm sediment section.

Unsaturated alcohols, consisting of two $C_{16:1}$ alkenols and up to three $C_{18:1}$ alkenols, were detected in the C. hypolimnica and both free and bound sediment samples; additionally a $C_{24:1}$ alkenol was detected in the C. hypolimnica sample. The C. hypolimnica sample contained the highest relative abundances of alkenols, with the first eluting $C_{16:1}$ isomer being the most abundant component of the alcohol fraction of this sample.

Hexadecan-2-ol, present in the lipid extracts of Priest Pot

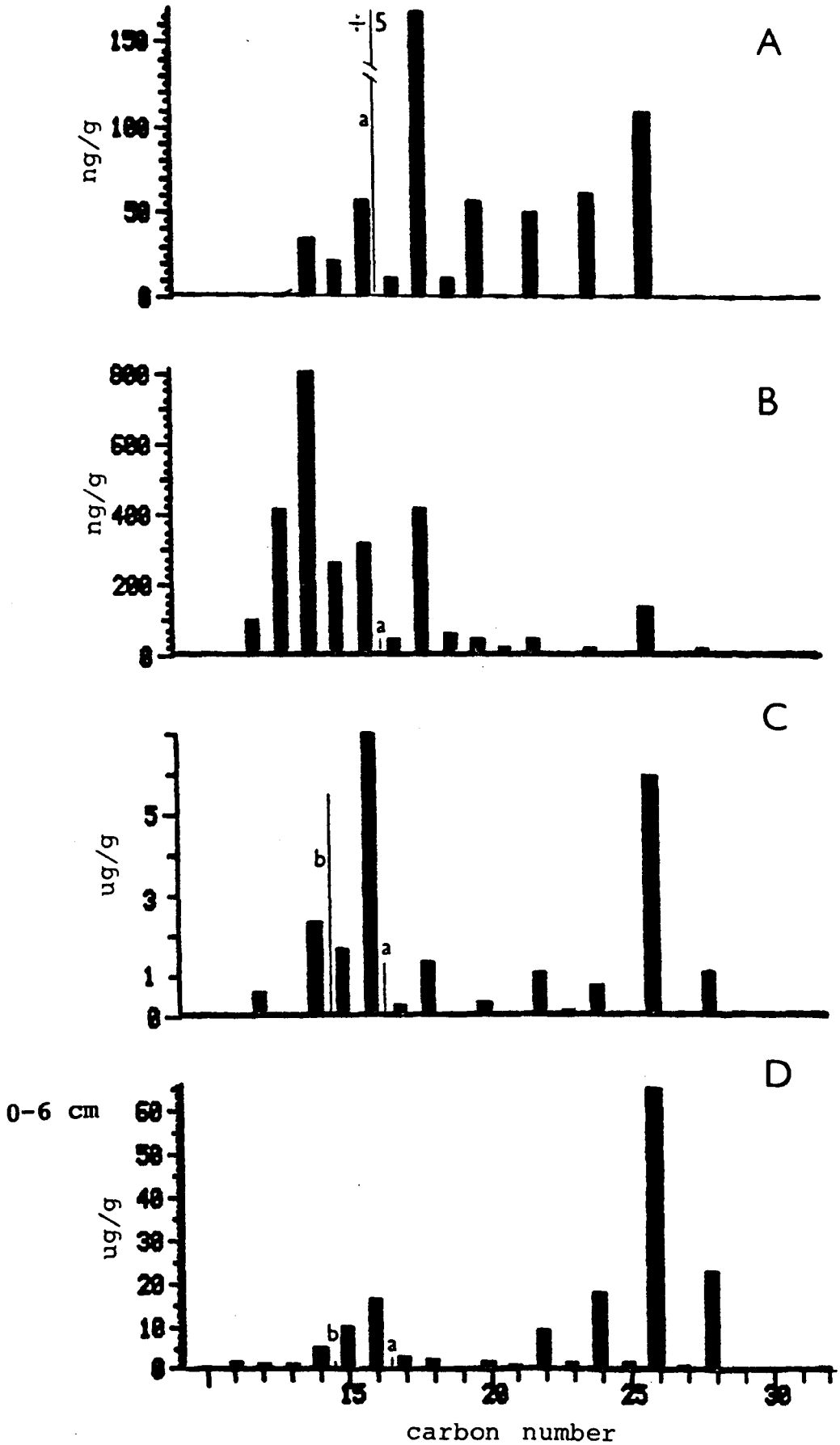


Fig. 4.2/6. Abundances of n-alkanols in: (A) rotifer total lipids; (B) ciliated protozoa total lipids; (C) Clathlochloris hypolimnica free lipids; (D) 0-6 cm free lipids. a = hexadecan-2-ol; b = farnesol (I)

Quantitation expressed as: A and B ng l water^{-1}, C ug l water^{-1}, D ug g dry, extracted sediment^{-1}, determined by comparison of GC peak areas with that of a known amount of n-C_{18} alkanol, all as TMS ethers.

In (A) "÷ 5" indicates that the abundance of hexadecan-2-ol is 5x greater than shown.

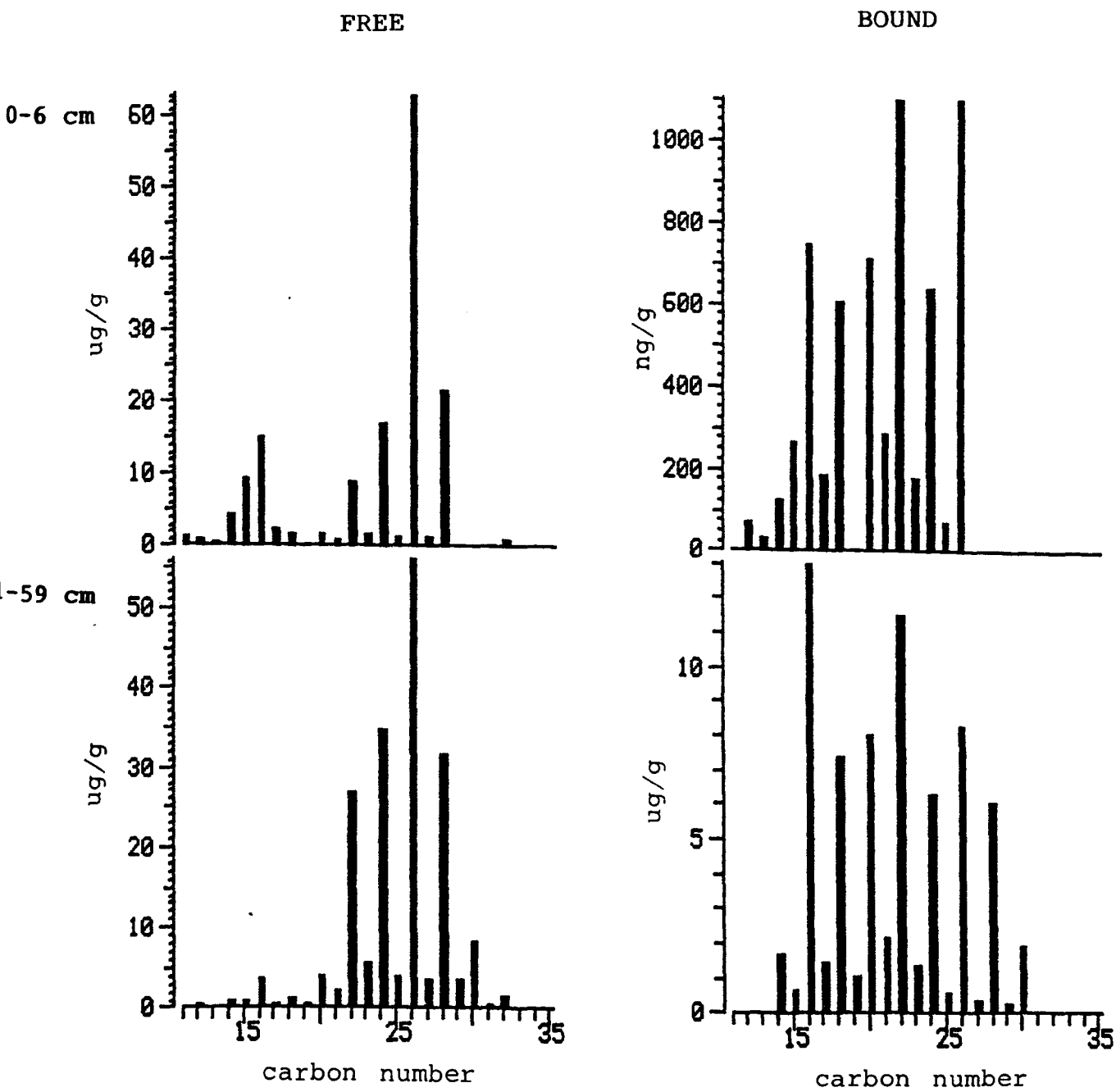


Fig. 4.2/7. Abundances of free and bound n-alkanols in Priest Pot 0-6 cm and 51-59 cm sediment sections.
Quantitation expressed as ng (or ug) g dry, extracted sediment^{-1}, determined by comparison of GC peak areas with that of a known amount of n-C_{28} alkanol, all as TMS ethers.

samples, has a chiral centre. Optically pure standards were not available for stereochemical analysis of the natural hexadecan-2-ol ; therefore, it was determined to separate the enantiomers of racemic hexadecan-2-ol by chromatography of diastereomeric derivatives produced by esterification with 2S-acetyl mandelic acid (see Chapter 8). HPLC analysis of the diastereomers of 2´-hexadecyl-2S-acetylmandelate produced a chromatogram with two well resolved peaks (Fig. 4.2/8). The diastereomers were isolated by preparative HPLC, with some loss of resolution being experienced when larger amounts of sample were chromatographed. Hexadecan-2-ol was released by hydrolysis and the stereochemistry assigned on the basis of measured optical rotations compared with reported data for octan-2-ol and eicosan-2-ol (Serk-Hanssen et al., 1953). Thus, hydrolysis of the first eluting HPLC fraction (A) produced hexadecan-2R-ol and hydrolysis of the second eluting HPLC fraction (B) produced hexadecan-2S-ol. For the purposes of GC, hexadecan-2-ol was derivatised with R-trans-chrysanthemic acid (Brooks et al.,1973). GC analysis performed on a 25 m OV1 coated Flexsil column and an open tubular glass column (56 m x 0.24 mm i.d.) wall-coated with DEGS/PEGS liquid phase (3:1; 0.06 um phase thickness) previously described by Maxwell et al. (1980), showed that enriched fractions had been produced (Fig. 4.2/9), although optically pure hexadecan-2-ol could probably be obtained by the above method by injecting smaller amounts of sample onto the HPLC and taking careful "cuts". Fraction A was found to contain 75% hexadecan-2R-ol and fraction B contained 80% hexadecan-2S-ol. The elution order of the R-trans-chrysanthemic acid esters was

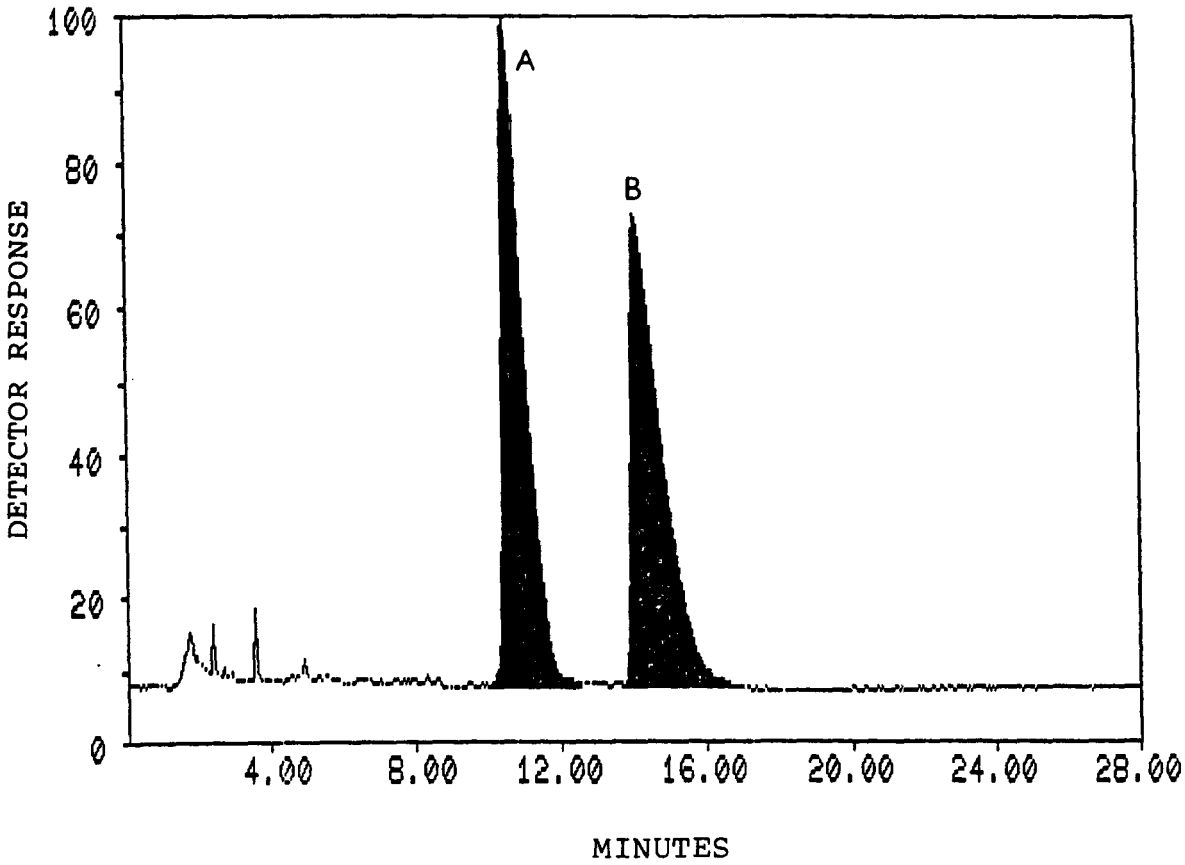


Fig. 4.2/8. Liquid chromatogram of the mixture of diastereomeric esters (A and B) prepared from racemic hexadecan-2-ol and S-acetylmandelic acid.
A = acetylmandelate of hexadecan-2R-ol;
B = acetylmandelate of hexadecan-2S-ol.

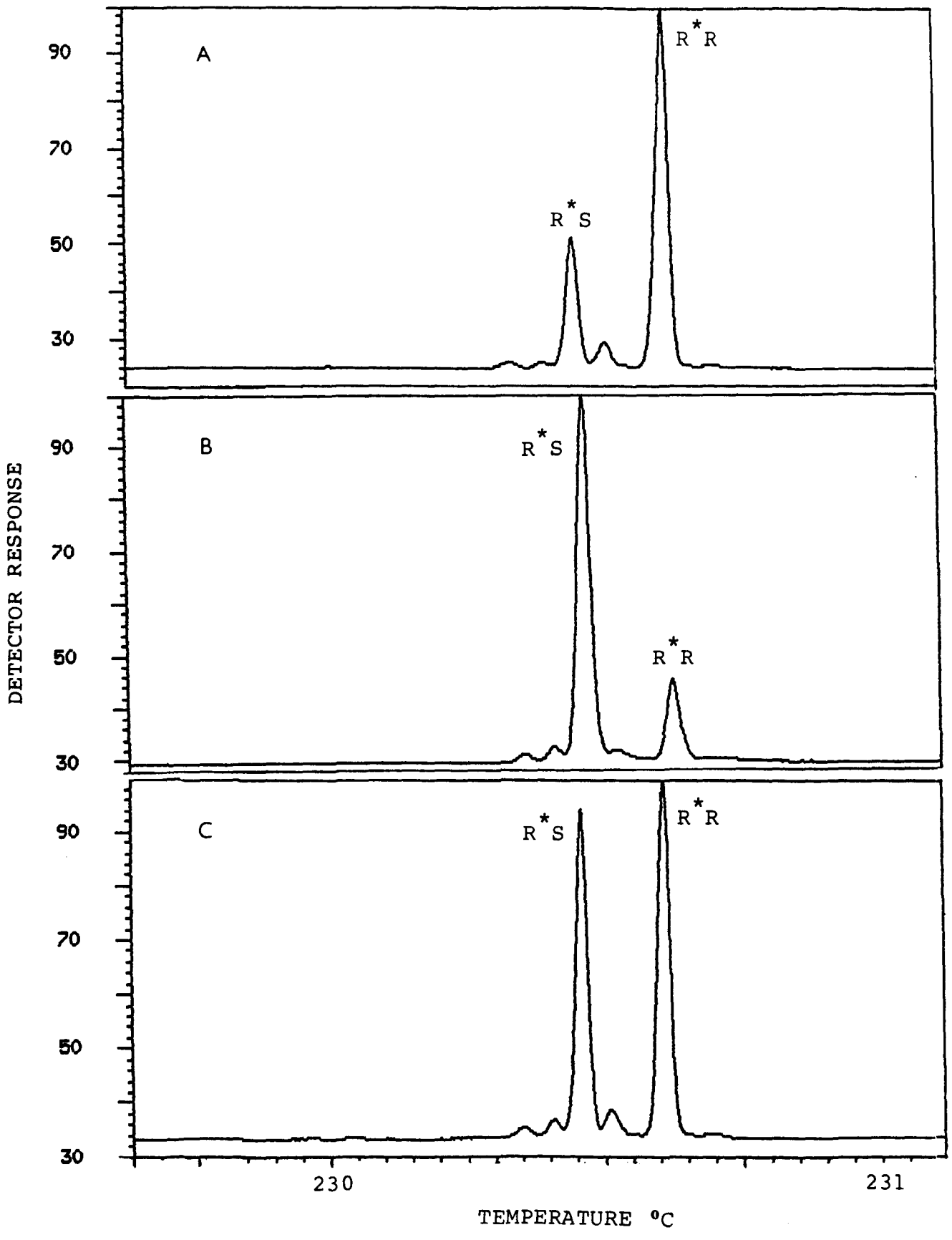


Fig. 4.2/9. Partial gas chromatogram of R-trans-chrysanthemate esters of hexadecan-2-ol from: (A) HPLC fraction A; (B) HPLC fraction B; (C) HPLC fractions A and B combined. Peaks assigned from optical rotations (see text), * refers to derivatising agent. Small peaks are due to the presence of low levels of isomers of R-trans-chrysanthemic acid.
GC conditions: 25m x 0.30mm Flexsil column coated with OV1 liquid phase , 60-270 C at 4 min^{-1}, H_2 carrier.

established as SR before RR on both OV1 and DEGS/PEGS liquid phases. The optically enriched standards were used to determine the enantiomeric composition of natural hexadecan-2-ol in the rotifers, ciliated protozoa, C. hypolimnica and surficial free and bound lipids, by GC and GC-MS coinjections derivatised as esters of R-trans-chrysanthemic acid; results are presented in Table 4.2/5.

b) Cyclic alcohols

Complex sterol distributions, with many coeluting components were observed. Extensive use of mass fragmentography was used to interpret the mass spectra. Sterol distributions are given in Fig. 4.2/10 and compounds are tabulated in Table 4.2/6. Compounds labelled 11 and 26 in Fig. 4.2/10 were found to elute at the front of peaks also containing compounds labelled 12 and 27 respectively. Mixed mass spectra, therefore, resulted, but on the basis of the molecular ions, loss of 90 amu from these ions and a base peak at m/z 251, C_{28} and C_{29} sterols with $\Delta^{5,7,9(11),22}$ unsaturation were inferred.

C_{30} and C_{32} hopanols, mainly having the 17β(H),21β(H) stereochemistry, were identified amongst the sedimentary alcohol fractions, with the highest relative abundance occurring in the free lipids of the 51-59 cm section. Olean-12-en-3β-ol (XXI) and urs-12-en-3β-ol (XXII) were detected in relatively low amounts amongst the sedimentary alcohol fractions.

c) Alkandiols

C_{30} and C_{32} alkan-1,15-diols were identified from their mass spectra amongst the free and bound alcohols of the 51-59 cm section. The C_{30} alkan-1,15-diol (TMS) present in the free

Table 4.2/5 Enantiomeric composition[a] of a hexadecan-2-ol in Priest Pol samples

Sample		R : S Ratio
Rotifers	(Total)	0.37
Ciliated protozoa	(Total)	0.43
Clathrochloris	(Free)	4.88
0-6 cm Sediment	(Free)	2.70
0-6 cm Sediment	(Bound)	1.33

[a]Determined by GC coinjection with optically enhanced standards derivatised as esters of (+)-trans-chrysanthemic acid and by GC-MS.

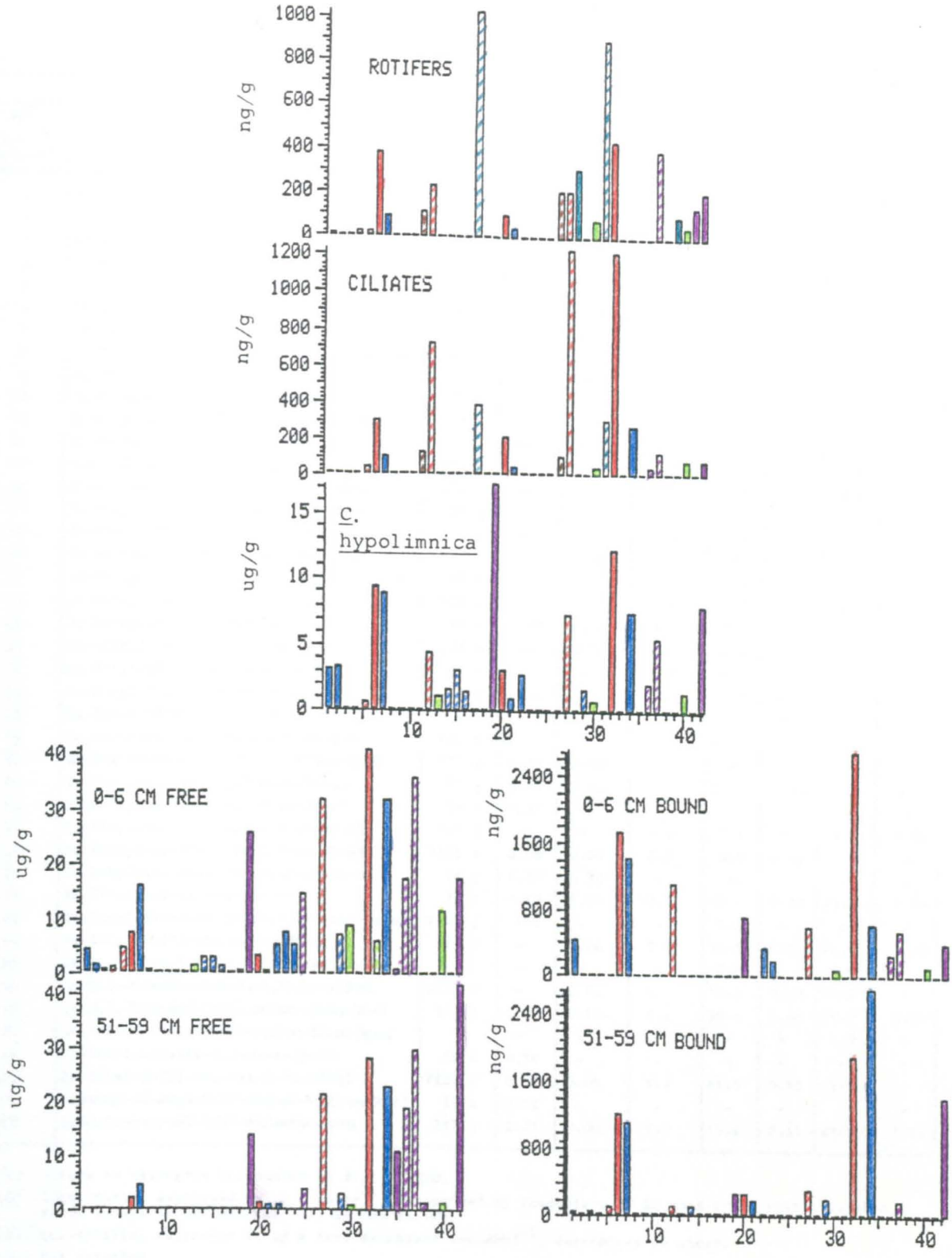


Fig. 4.2/10. Distribution of sterols in Priest Pot samples. Compound identifications and quantitations are fully explained in Table 4.2/6.

Δ^{0} – Δ^{7} –

Δ^{5} – $\Delta^{8(14)}$ –

$\Delta^{5,7}$ – 4 Me –

$\Delta^{5,7,9(11)}$ – Hatching indicates presence of Δ^{22}

Table 4.2/6 Sterol abundances in Priest Pot samples

Histogram bar No.(a) (Fig.4.2/10)	Name	Structure	Rotifers µg/l(b)	Ciliated protozoa µg/l(b)	Clathrocloris µg/l(c)	0 - 6 cm		51 - 59 cm	
						Free µg/g(c)	Bound µg/g(c)	Free µg/g(c)	Bound µg/g(c)
1	5β(H)-Cholestan-3β-ol	II a	0.01	-	3.0	4.1	0.43	0.3	0.05
2	5α(H)-Cholestan-3α-ol	IV a	-	-	3.2	1.4	tr.	0.15	tr.
3	5β(H)-Cholestan-3α-ol	V a	-	-	-	0.6	tr.	0.15	0.05
4	27-Nor-24-methylcholesta-5,22-dien-3β-ol	VI b	0.02	-	-	0.8	tr.	-	-
5	Cholesta-5,22-dien-3β-ol	VI c	0.02	0.04	0.6	4.3	tr.	-	0.15
6	Cholest-5-en-3βol	VI a	0.37	0.30	9.1	7.1	1.72	2.2	1.20
7	5α(H)-Cholestan-3β-ol	III a	0.09	0.10	8.6	15.7	1.40	4.5	1.10
8	27-Nor-24-methyl-5α(H)-cholestan-3β-ol	III d	-	-	-	0.6	-	-	-
9	24-Methyl-5β(H)-cholestan-3β-ol	II e	-	-	-	0.3	-	-	-
10	24-Methyl-5α(H)-cholestan-3α-ol	IV e	-	-	-	0.2	-	-	-
11	24-Methylcholesta-5,7,9(11),22-tetraen-3β-ol	VII f	0.11	0.12	-	-	-	-	-
12	24-Methylcholesta-5,22-dien-3β-ol	VI f	0.23	0.72	4.3	0.3	1.09	0.1	0.10
13	5α(H)-Cholest-7-en-3β-ol	VIII a	-	-	1.1	1.5	-	tr.	-
14	24-Methyl-5α(H)-cholest-22-en-3β-ol	III f	-	-	1.5	3.0	-	0.2	0.10
15	24-Ethyl-5β(H)-cholest-22-en-3β-ol	II g	-	-	3.0	3.0	-	-	-
16	24-Ethyl-5β(H)-cholest-22-en-3α-ol	V g	-	-	1.4	1.5	-	-	-
17	24-Methylcholesta-5,7,22,-trien-3β-ol	IX f	1.02	0.38	-	tr.	-	-	-
18	4α-Methyl-5α(H)-cholest-8(14)-en-3β-ol	XI a	-	-	-	0.4	-	tr.	-
19	4α-Methyl-5α(H)-cholestan-3β-ol	XII a	-	-	17.0	25.3	-	13.5	0.25
20	24-Methylcholest-5-en-3β-ol	VI e	0.10	0.20	3.0	3.2	0.72	1.3	0.25
21	24-Methyl-5α(H)-cholestan-3β-ol	III e	tr.	0.04	0.9	0.5	-	0.9	0.16
22	24-Ethyl-5β(H)-cholestan-3β-ol	II h	tr.	-	2.7	5.3	0.36	1.0	-
23	24-Ethyl-5α(H)-cholestan-3α-ol	IV h	tr.	-	-	7.7	0.20	-	-
24	24-Ethyl-5β(H)-cholestan-3α-ol	V h	-	-	-	5.2	-	-	-
25	4α,24-Dimethyl-5α(H)-cholest-22-en-3β-ol	XII f	-	-	-	14.4	-	3.6	-
26	24-Ethylcholesta-5,7,9(11),22-tetraen-3β-ol	VII g	0.21	0.10	-	-	-	-	-
27	24-Ethylcholesta-5,22-dien-3β-ol	VI g	0.21	1.22	7.2	31.4	0.60	21.3	0.30
28	24-Methylcholesta-5,7-dien-3β-ol	IX e	0.31	-	-	-	-	-	-
29	24-Ethyl-5α(H)-cholest-22-en-3β-ol	III g	-	-	1.6	7.1	-	2.9	0.20
30	24-Methyl-5α(H)-cholest-7-en-3β-ol	VIII e	0.08	0.04	0.8	8.8	0.08	1.1	-
31	24-Ethylcholesta-5,7,22-trien-3β-ol	IX g	0.89	0.30	-	tr.	-	-	-
32	24-Ethylcholest-5-en-3β-ol	VI h	0.43	1.20	12.1	40.7	2.72	27.9	1.90
33	24-Ethyl-5α(H)-cholesta-7,22-dien-3β-ol	VIII g	tr.	-	-	6.2	-	-	-
34	24-Ethyl-5α(H)-cholestan-3β-ol	III h	-	0.26	7.4	31.5	0.63	22.8	2.70
35	4α,24-Dimethyl-5α(H)-cholestan-3β-ol		-	-	-	0.8	-	11.0	-
36	4α,23,24-Trimethylcholesta-5,22-dien-3β-ol	XIII i	-	0.04	2.1	17.4	0.28	18.0	-
37	4α,23,24-Trimethyl-5α(H)-cholest-22-en-3β-ol	XII i	0.39	0.12	5.4	35.4	0.56	29.7	0.18
38	4α-Methyl,24-ethyl-5α(H)-cholest-22-en-3β-ol		-	-	-	-	-	1.2	-
39	24-Ethylcholesta-5,7-dien-3β-ol	IX h	0.10	-	-	-	-	-	-
40	24-Ethyl-5α(H)-cholest-7-en-3β-ol	VIII h	0.05	0.08	1.4	11.5	0.12	1.2	-
41	4α-Methyl-24-ethyl-5α(H)-cholest-8(14)-en-3β-ol	XI h	0.14	-	-	-	-	-	-
42	4α,23,24-Trimethyl-5α(H)-cholestan-3β-ol	XII j	0.21	0.08	7.9	17.4	0.41	41.6	1.40

(a) Refers to histogram bar number in Fig. 4.2/10.

(b) Quantitation expressed as µg l water^{-1}, determined by comparison of GC peak areas with that of a known amount of cholesterol, all as TMS ethers.

(c) Quantitation expressed as µg g dry, extracted sediment^{-1}, determined as above.

(N.D.) Not detected

(tr.) Trace levels present.

fraction coeluted with the 1,14- and 1,13-diol isomers and with C_{30} alkan-15-one-1-ol (TMS) (see section 6.2.iii).

4.2.iv Ketones

6,10,14-Trimethylpentadecan-2-one occurred in the rotifers, C. hypolimnica, 0-6 cm free lipids and 51-59 cm bound lipids. The ketone fraction of the ciliated protozoa showed essentially one GC peak which had a mass spectrum very similar to that of a phytadiene, suggesting the compound producing this peak might be an aldehyde produced by oxidation of phytol which undergoes dehydration in the mass spectrometer source. Straight chain alkan-2-ones with a high CPI were identified in the free lipids of the 0-6 cm sediment and the free and bound lipids of the 51-59 cm sediment (Fig. 4.2/11).

Steroidal ketones were present in the free lipids of C. hypolimnica and the sediment sections; abundances are given in Table 4.2/7. The bound lipids of the 51-59 cm section contained C_{27}, C_{28} and C_{29} $\Delta^{3,5}$ ster-7-ones (120,70 and 410 ng/g respectively) as well as trace amounts of C_{27}-C_{29} stanones and Δ^4 stenones. Hopanoid ketones were present at low levels amongst the bound lipids of the 51-59 cm sediment section, but were relatively more abundant in C. hypolimnica and the sedimentary free lipids, which also contained higher plant derived triterpenoid ketones (Table 4.2/8).

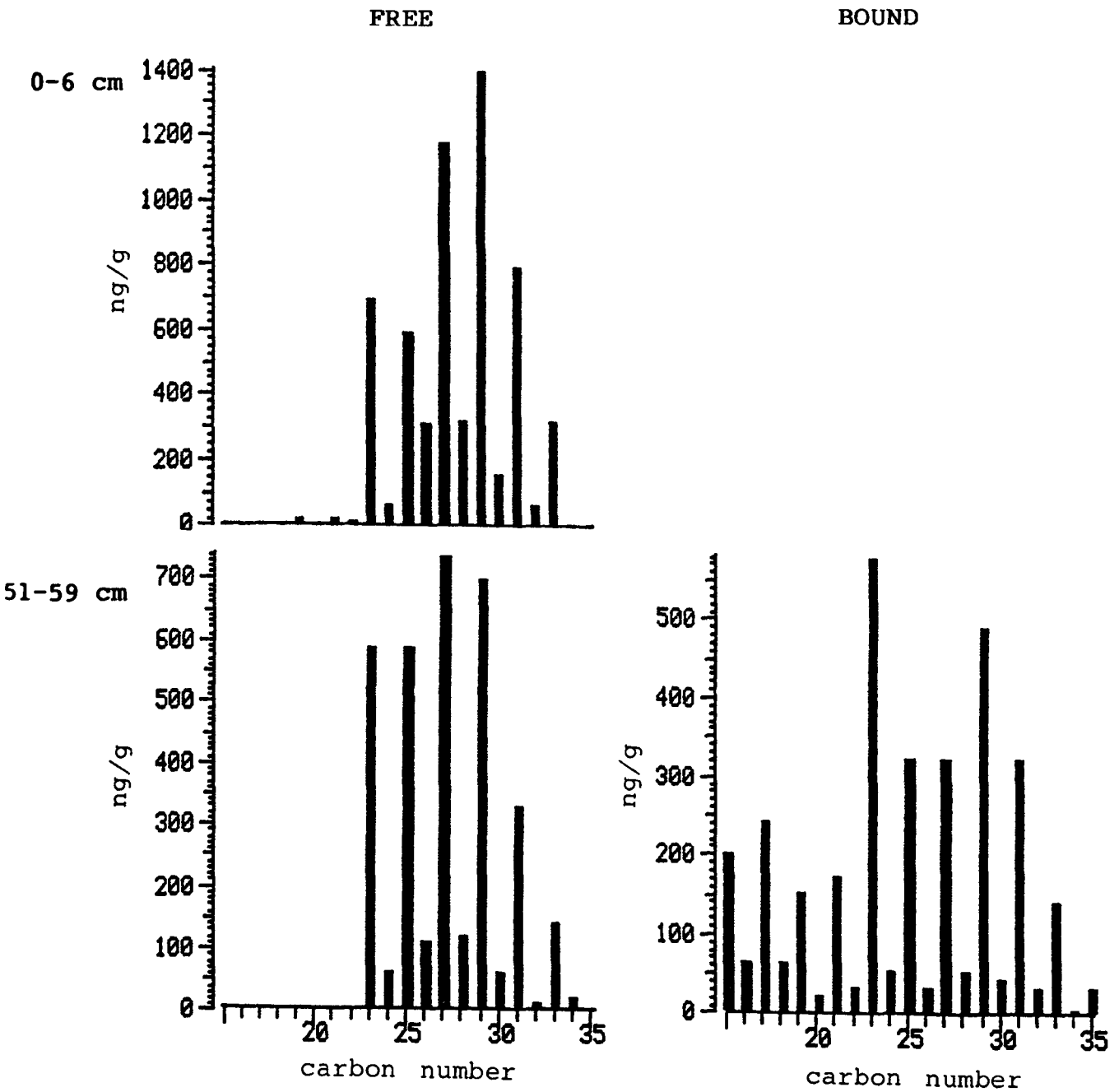


Fig. 4.2/11. Abundances of free and bound alkan-2-ones in Priest Pot 0-6 cm and 51-59 cm sediment sections. Bound alkan-2-ones were not detected in the 0-6 cm section. Quantitation expressed as ng g dry, extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of $\underline{n}$-C_{18} and $\underline{n}$-C_{28} alkanes.

Table 4.2/7 Abundances of free steroidal ketones in Priest Pot samples

Compound	Structure	Clathrochloris µg l^{-1} (a)	0 - 6 cm µg g^{-1} (b)	51 - 59 cm ng g^{-1} (c)
5β(H)-Cholestan-3-one	XIV a	0.24	0.4	tr.
5α(H)-Cholestan-3-one	XV a	2.12	0.8	10
24-Methyl-5α(H)-cholest-22-en-3-one	XV f	N.D.	0.1	N.D.
24-Methyl-5β(H)-cholestan-3-one	XIV e	N.D.	N.D.	tr.
4α-Methyl-5α(H)-cholestan-3-one	XVI a	0.68	1.8	70
4α,24-Dimethyl-5α(H)-cholest-22-en-3-one	XVI f	0.32	2.5	50
24-Methyl-5α(H)-cholestan-3-one	XV e	N.D.	0.6	tr.
24-Ethyl-5β(H)-cholestan-3-one	XIV h	0.22	0.8	10
4α,24-Dimethyl-5α(H)-cholestan-3-one	XVI e	0.16	0.5	90
4α,23,24-Trimethyl-5α(H)-cholest-22-en-3-one	XVI i	1.86	5.9	500
24-Ethyl-5α(H)-cholestan-3-one	XV h	N.D.	0.9	100
4α,23,24-Trimethyl-5α(H)-cholestan-3-one	XVI j	0.41	2.2	270

(a) Quantitation expressed as µg l water^{-1}, determined by comparison of GC peak areas with that of a known amount of n-C_{28} alkane.

(b) Quantitation expressed as µg g dry, extracted sediment^{-1}, determined as above.

(c) Quantitation expressed as ng g dry, extracted sediment^{-1}, determined as above.

(N.D.) Not detect.

(tr.) Trace levels present.

Table 4.2/8 Abundances of triterpenoidal ketones in Priest Pot sediments

Compound	Structure	Clathrochloris ($ng\ l^{-1}$)[a]	0 - 6 cm Free ($ng\ g^{-1}$)[b]	51- 59 cm Free ($ng\ g^{-1}$)[b]
Hopanoids				
22,29,30-Trisnorhopan-21-one	XL	10	240	30
30-Norhopan-22-one	XLI	N.D.[c]	N.D.	60
Hopanone	XLII	tr.[d]	340	250
Bishomohopanone		N.D.	20	N.D.
Trishomohopanone		N.D.	30	20
Higher plant triterpenoids				
Taraxer-14-en-3-one	XXIV	N.D.	140	410
Lup-22(29)-en-3-one[e]		N.D.	100	N.D.
Olean-12-en-3-one	XXV	150	470	140
Urs-12-en-3-one	XXVI	100	70	150
Lupan-3-one	XXVIII	N.D.	80	860
Friedelan-3-one	XXIX	N.D.	800	1 100

(a) Quantitation expressed as ng l water^{-1}, determined by comparison of GC peak areas with that of a known amount of n-C_{28} alkane.

(b) Quantitation expressed as ng g dry, extracted sediment^{-1}, determined as above.

(c) Not detected.

(d) Trace levels present.

(e) Tentative assignment based solely on mass spectral interpretation.

4.2.v Carboxylic acids

n-Alkanoic acids were detected in all samples; their distributions are shown in Figs. 4.2/12 and 4.2/13. Iso- and anteiso-branched fatty acids, mainly i-C_{13-17} and ai-$C_{13,15,17}$, were present in each sample, with a higher relative abundance in the C. hypolimnica and sedimentary bound lipid fractions. Phytanic acid was a significant component of the C. hypolimnica and 0-6 cm free acids. The bound lipids of the 51-59 cm sediment contained a series (C_{10}-C_{18}) of monounsaturated fatty acids, having a high even carbon number predominance, which eluted after the corresponding n-alkanoic acid as methyl esters on OV1 liquid phase. The mass spectra exhibited $M^{\cdot+}$, M-31, M-74 and a base peak of m/z 87, suggesting they might possess a methyl branch at the 4 position.

Trace levels of 17β(H),21β(H)-bishomohopanoic acid were identified amongst the free and bound lipids of the 0-6 cm sediment section, relatively higher amounts (300 ng/g) were detected in the bound acids of the 51-59 cm sediment, together with the 17α(H),21β(H)- and 17β(H),21α(H)-stereoisomers (70 and 40 ng/g respectively).

Alkenoic acids were present in all samples, their abundances are given in Table 4.2/9. C_{16}-C_{28} dicarboxylic acids were detected in the sedimentary bound lipid fractions, having a strong even carbon number predominance and maxima at C_{16} and C_{22}. They were more abundant in the deeper sediment sample, in which

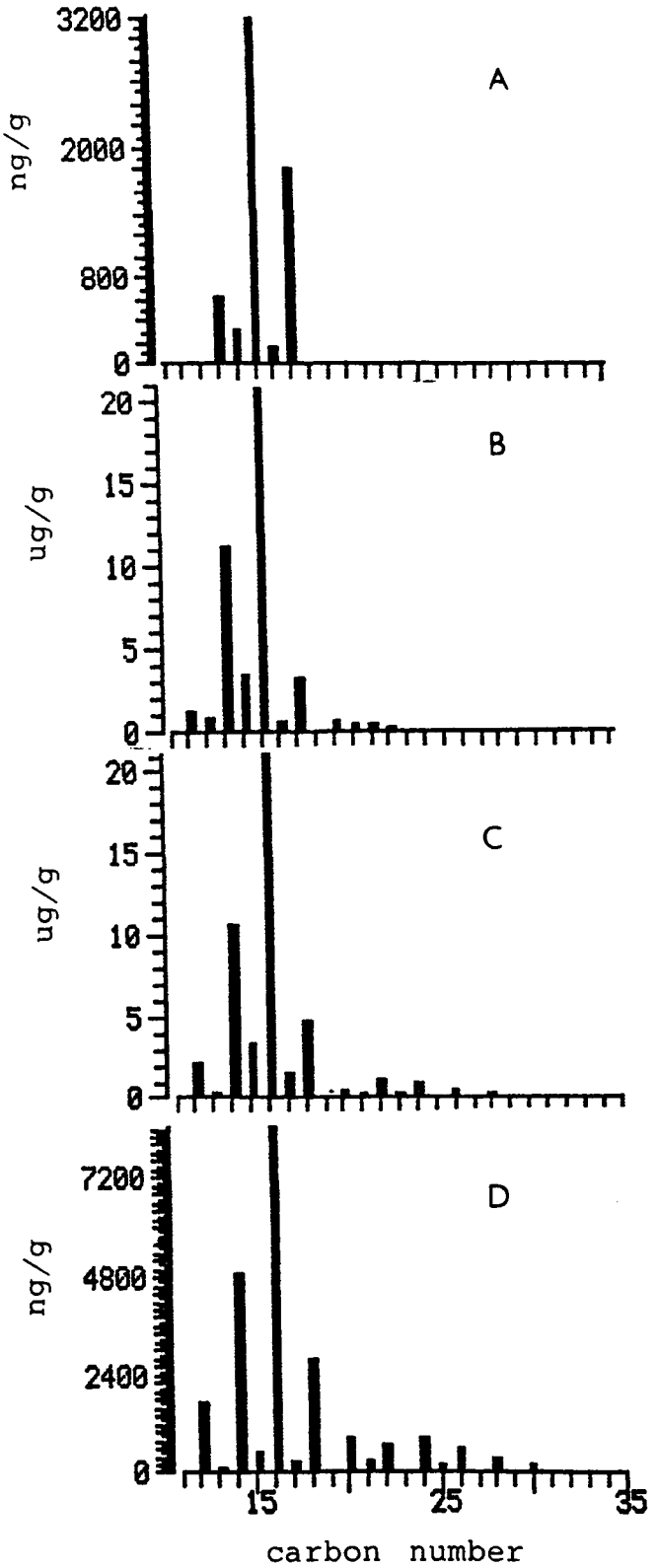


Fig. 4.2/12. Abundances of n-alkanoic acids in: (A) rotifer total lipids; (B) ciliated protozoa total lipids; (C) Clathrochloris hypolimnica free lipids; (D) 0-6 cm free sedimentary lipids.

Quantitation expressed as: A ng l water^{-1}, B and C ug l water^{-1}, D ng dry, extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of n-C_{18} and n-C_{28} alkanes.

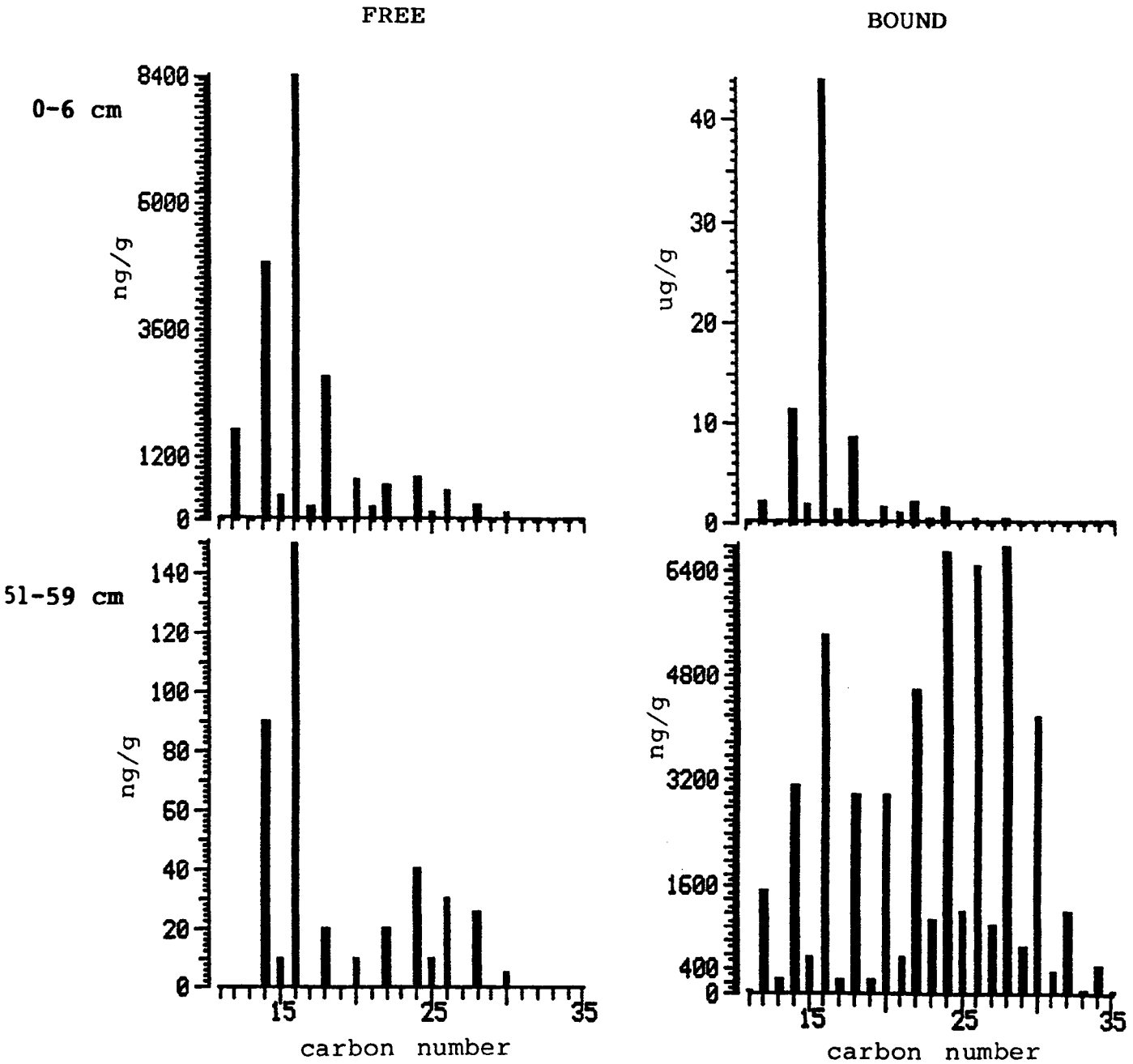


Fig. 4.2/13. Abundances of free and bound fatty acids in Priest Pot 0-6 cm and 51-59 cm sediment sections.
Quantitation expressed as ng (or ug) g dry, extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of $\underline{n}$-C_{18} and $\underline{n}$-C_{28} alkanes.

Table 4.2/9 Abundances of alkenoic acids in Priest Pot samples

Alkenoic acid[a]	Rot. ng/l[b]	Cil. ng/l[b]	Clath. ng/l[b]	0-6 cm Free ng/g[c]	0-6 cm Bound ng/g[c]	51-59 cm Free ng/g[c]	51-59 Bound ng/g[c]
14:3	-	80	-	-	-	-	-
14:1	-	40	890	-	-	-	-
15:1	-	-	1 700	-	-	-	-
16:4	-	5 400	-	-	-	-	-
16:2	-	1 100	-	-	-	-	-
16:3	-	1 200	-	-	-	-	-
16:1	-	300	-	1 200	12 800	80	-
16:1	160	6 000	23 800	120	-	-	-
16:1	-	140	-	130	1 900	-	-
18:3	-	-	-	400	-	-	-
18:2	-	8 100	-	-	-	-	-
18:2	-	-	-	} 1 100	3 800	-	-
18:3	-	10 800	-		4 700	-	-
18:1	160	10 000	3 900	3 200	8 800	-	400
18:1	-	6 000	-	-	5 200	-	100
20:4	-	4 200	-	-	-	-	-
20:4	-	460	-	-	-	-	-
20:3	-	1 900	-	-	-	-	-
20:1	-	1 000	-	90	-	-	-
20:1	-	200	-	-	-	-	-

- Not detected

(a) Position of double bonds not determined

(b) Quantitation expressed as ng l water^{-1}, determined by comparison of GC peak areas with a standard of n-C_{28} alkane.

(c) Quantitation expressed as ng g dry, extracted sediment^{-1}, determined as above.

higher carbon number components formed a greater proportion of the total.

4.2.vi Wax esters

Wax esters were isolated from the free lipids of the two sediment sections. They had a high even carbon number predominance and maximised at C_{44}; molecular compositions are given in Table 4.2/10. Steryl esters were also present, the following compounds were recognised in the fraction isolated from the 51-59 cm section:

cholesterol-16:0; 18:1; 18:0

cholestanol-16:0

24-methylcholesterol-16:0

24-methylcholestanol-15:0

24-ethylcholesta-5,22-dienol-16:0

24-ethylcholesterol-15:0; 16:0

Additionally a phytol-18:0 ester was present.

C_{31} and C_{33} mid-chain ketones were detected amongst the wax ester fraction of the 51-59 cm sediment sample.

Table 4.2/10 Molecular composition of wax esters in Priest Pot sediments

Compound[a]	Alcohol - Acid[b]		% [c] 0 - 6 cm	% [c] 51 - 59 cm[d]
n 26	16	10	N.D.	74
	14	12	N.D.	26
i 28	18	10	tr.	95
	16	12	tr.	5
n 28	18	10	N.D.	73
	17	11	N.D.	14
	16	12	53	13
	15	13	25	N.D.
	14	14	22	N.D.
n 29	17	12	8	N.D.
	16	13	28	N.D.
	15	14	32	N.D.
	14	15	15	100
	13	16	17	N.D.
n 30	17	13	7	9
	16	14	64	64
	15	15	15	9
	14	16	14	12
	12	18	N.D.	5
n 31	17	14	19	(d)
	16	15	41	
	15	16	31	
	14	17	5	
	13	18	4	
n 32	18	14	18	10
	17	15	10	21
	16	16	72	63
	15	17	N.D.	6
n 33	18	15	22	(d)
	17	16	42	
	16	17	25	
	15	18	11	
n 34	18	16	91	40
	17	17	N.D.	15
	16	18	7	39
	14	20	2	6
n 36	20	16	23	18
	18	18	63	68
	16	20	9	14
	15	21	1	N.D.
	14	22	4	N.D.
n 38	24	14	N.D.	5
	22	16	47	42
	20	18	34	25
	18	20	11	20
	16	22	8	9
n 40	26	14	12	4
	24	16	37	33
	22	18	23	17
	20	20	21	37
	18	22	7	5
	16	24	N.D.	4
n 42	26	16	62	35
	24	18	15	14
	22	20	18	35
	20	22	5	9
	18	24	N.D.	4
	16	26	N.D.	3

Continued....

Table 4.2/10 Molecular composition of wax esters in Priest Pot sediments - continued.

Compound[a]	Alcohol - Acid[b]		% [c] 0 - 6 cm	% [c] 51 - 59 cm[d]
n 44	28	16	9	21
	26	18	80	49
	24	20	5	12
	22	22	6	18
n 46	30	16	4	
	28	18	41	
	26	20	33	
	24	22	14	
	22	24	8	
n 48	30	18	25	
	28	20	13	
	26	22	52	
	24	24	8	
	22	26	2	

(a) Short-hand notation is used to give information on branching pattern followed by total number of carbons.

(b) Molecular composition. First number is the alkyl chain length, second number is the acyl chain length.

(c) Different alcohol-acid pairings of the same total carbon number and branching pattern coeluted in GC. % molecular compositions were determined from the relative proportions of $(RCO_2H)^{\cdot+}$ fragments in the mass spectrum, obtained by summing over the whole peak.

(d) Odd carbon number compounds were of low abundance, the ensuing poor quality spectra did not allow for reliable determination of molecular compositions.

4.2.vii Hydroxy acids

Hydroxy acids were not detected in the lipids of the organisms. The free lipids of the 0-6 cm sediment contained relatively low amounts (<150 ng/g) of C_8-C_{28} 3-hydroxy acids maximising at n-C_{14} and containing a high proportion of iso- and anteiso-branched species; C_{16}-C_{28} 2-hydroxy, w-hydroxy and (w-1)-hydroxy acids were present at trace levels. 3-Hydroxy acids were not detected amongst the free lipids of the 51-59 cm sediment section; C_{14}-C_{28} even carbon number w-hydroxy acids were present, maximising at C_{16} and C_{22} (80 ng/g each). Hydroxy acids were relatively abundant constituents of the bound lipids of the sediment samples. The distributions of bound 3-hydroxy acids is shown in Fig. 4.2/14A and those of bound w-hydroxy acids in Fig. 4.2/14B. C_{18}-C_{26} 2-hydroxy acids maximising at C_{24} (880 ng/g) were detected in the bound hydroxy acid fraction of the 0-6 cm sediment. Although they coeluted with the 3-hydroxy acid isomers when analysed by GC as methyl ester, TMS ethers on OV1 liquid phase, relative proportions could be estimated from the mass spectra (Eglinton et al., 1968). Trace levels only of C_{20}-C_{30} 2-hydroxy acids were present amongst the bound lipids of the 51-59 cm section. Relatively low levels of C_{26}, C_{28} and C_{30} (w-1)-hydroxy acids were detected in the 0-6 cm bound lipids.

4.2.viii Aldehydes

The free lipids of the 0-6 cm sediment section contained C_{19}-C_{28} saturated aldehydes and C_{20}, C_{22} and C_{24} polyunsaturated

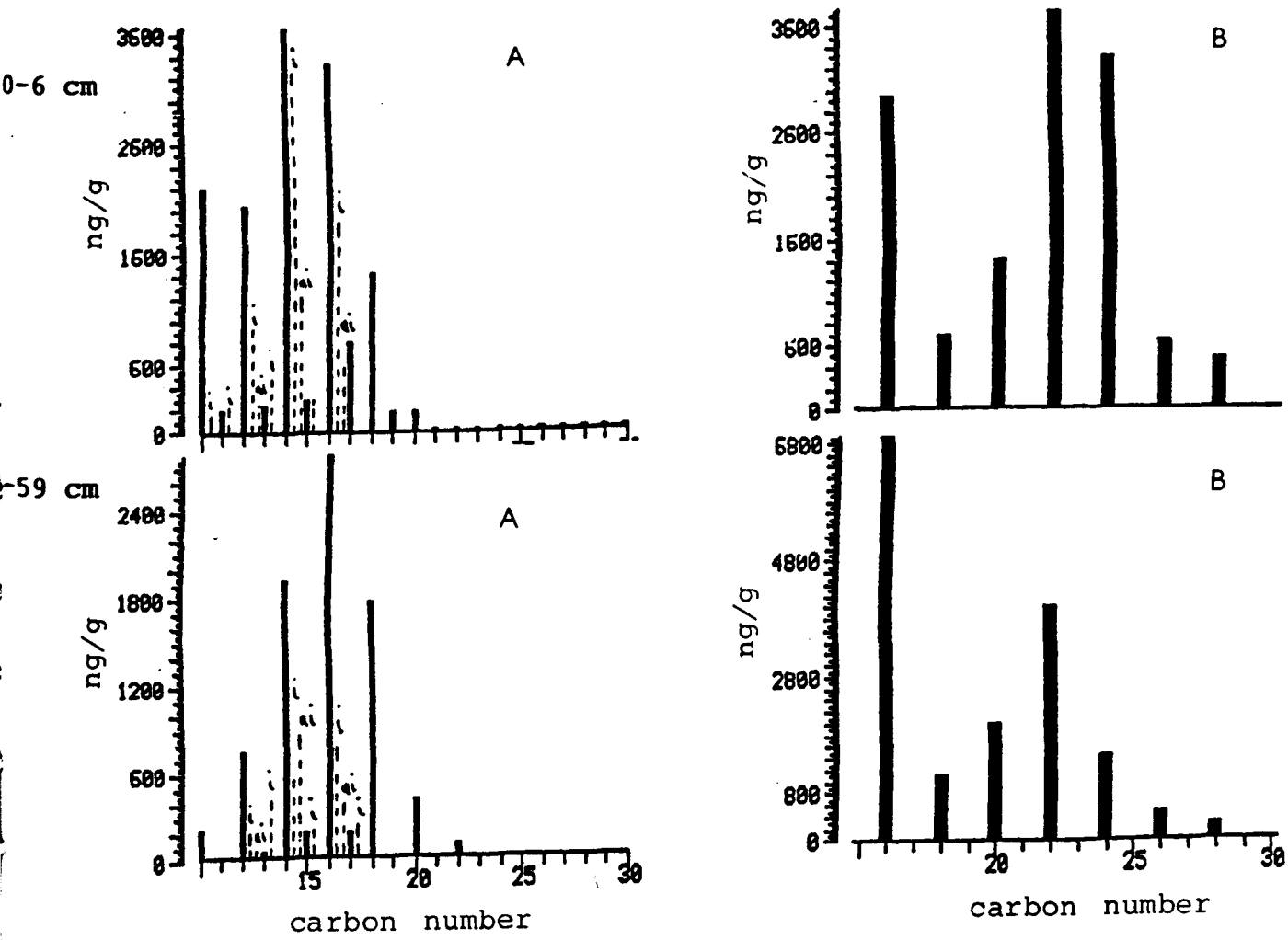


Fig. 4.2/14. Abundances of sedimentary bound:
(A) 3-hydroxy acids
(B) w-hydroxy acids.
Quantitation expressed as ng g dry, extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of $\underline{n}$-C_{18} and $\underline{n}$-C_{28} alkanes.

aldehydes; abundances are given in Table 4.2/11.

4.3 DISCUSSION

The organisms and sediment samples all contained complex lipid assemblages. Discussion is arranged by compound type with the lipids of the organisms being discussed prior to those of the sediment.

4.3.i Acyclic alcohols

The rotifer, ciliated protozoa and C. hypolimnica samples have bimodal distributions of n-alkanols with the first maximum at C_{18}, C_{14} and C_{16} respectively; the second smaller maximum is at C_{26} in each case (Fig. 4.2/6). In the 0-6 cm sediment section, free n-alkanols show a bimodal distribution with maxima at C_{16} and C_{26}, indicating a mixed autochthonous and allochthonous input. Lower molecular weight homologues are relatively less abundant in the 51-59 cm section (Fig. 4.2/7), consistent with a more rapid degradation of shorter chain compounds in sediments (Quirk, 1978; Cranwell, 1981a). The relative abundance of the C_{20} n-alkanols in the rotifer sample is probably enhanced by the low absolute levels of n-alkanols, resulting in a greater relative contribution from higher plant particles; in the case of the C. hypolimnica and 0-6 cm free alkanols, preferential preservation of longer chain length alkanols derived from higher

Table 4.2/11 Abundances of aldehydes isolated from the free lipids of Priest Pot 0-6 cm sediment

Compound[a]	Abundance ($ng\ g^{-1}$)[b]
Saturated	
C_{19}	2
C_{20}	4
C_{21}	tr.
C_{22}	6
C_{23}	2
C_{24}	110
C_{25}	65
C_{26}	840
C_{27}	33
C_{28}	259
Unsaturated	
$C_{20:3}$	932
$C_{22:4}$	743
$C_{24:6}$	657

(a) Identifications based on mass spectral interpretation, $M^{\cdot+}$, M - 18, M - 44, m/z 96, m/z 82, m/z 68, m/z 57.

(b) Quantitation expressed as ng g dry, extracted sediment^{-1}, determined by comparison of GC peak areas with those of known amounts of n-C_{18} and n-C_{28} alkanes.

(tr.) Trace levels present

plants is apparent. The bound n-alkanols of the sediments maximise at C_{16}, C_{22} and C_{26} and have a relatively high proportion of $<C_{22}$ alkanols (Fig. 4.2/7), consistent with greater preservation of lower molecular weight compounds for bound lipids (Cranwell, 1981a). Bound alkanols were more abundant in the deeper sediment section, implying that formation of bound alkanols takes place within the sediment. The maximum at C_{22} may reflect activity of decomposer organisms in the sediment (Cranwell, 1981a). The presence of free C_{30} and bound C_{28} and C_{30} n-alkanols in the 51-59 cm section, which were not detected in the 0-6 cm section, suggests that there may have been a change in input between deposition of the two sediment layers.

Sedimentary iso- and anteiso-branched C_{15} alkanols have been proposed to originate from bacteria (Cranwell, 1980), the higher relative abundance of such compounds in the bound lipids than in the free, supports the view that bound lipids include a relatively large bacterial contribution (e.g. Brooks et al. 1976). The unidentified C_{20} diol, present in the bound lipid fraction of the 0-6 cm sediment, presumably originates from Peridinium lomnickii as it has been isolated from this organism (Chapter 3, this thesis), although in the organism it is present in a free state.

Recognition of monoene alcohols in the sediment and C. hypolimnica samples is consistent with what is known of bacterial lipids (Perry et al., 1979; Gillan et al., 1983). In the absence of determination of double bond positions, however, little more information may be derived from them. Farnesol is the esterifying alcohol in bacteriochlorophyll, known to be present

in Chlorobium bacteria (Rapoport and Hamlow, 1961), hence recognition of farnesol in the alcohol fraction of Priest Pot C. hypolimnica was expected. The detection of farnesol in the 0-6 cm sedimentary free lipids demonstrates an input of C. hypolimnica lipids and, as farnesol contains three double bonds, provides evidence for the greater preservation of labile compounds in anoxic sediments such as those of Priest Pot (Cranwell, 1982).

The detection of hexadecan-2-ol and trace amounts of the C_{17} and C_{18} alkan-2-ols in the rotifer and ciliate total lipids, the C. hypolimnica free lipids and the sediment free and bound lipids is interesting in view of their known occurrence. A series of alkan-2-ols (C_{16}-C_{20}) having exclusive 2S stereochemistry, detected in the bound lipids of contemporary and 9000 year old lake sediments, was attributed to the complex lipids of bacterial cell walls (Cranwell, 1980). There are few references to additional sources of alkan-2-ols. C_{18}-C_{20} alkan-2-ols of undefined stereochemistry have been found as esters in barley epicuticular wax (Wettstein-Knowles and Netting, 1976) and preening wax of the night heron (Jacob, 1975); they occur free in aerobically cultured Escherichia coli (Naccarato et al., 1972). Hexadecan-2-ol has not been reported as a constituent lipid of any algae. As the organisms from Priest Pot are natural populations and not axenic cultures the origin of the hexadecan-2-ol in them cannot be conclusively stated. Considering the amount present amongst the lipids of the rotifer sample, however, it seems likely that hexadecan-2-ol is a constituent of one or more of the rotifer species present in

Priest Pot. Although bacterial cell wall lipids may be providing an input of hexadecan-2-ol to the sediment, this is not the sole source as both S and R enantiomers are found (Table 4.2/5). The variation in the enantiomeric composition of hexadecan-2-ol in the rotifer, ciliate and C. hypolimnica samples and the 0-6 cm sedimentary free and bound lipids may reflect the respective R:S ratios of different sources or a difference in the degradation rates of the two enantiomers. If alkan-2-ols are present associated with the cell walls of C. hypolimnica they would be isolated from the bound lipids, the R:S ratio of such bound alkan-2-ols may well be different from that of the free hexadecan-2-ol.

4.3.ii Cyclic alcohols

The organisms and sediment samples contain a complex suite of sterols containing a high proportion of 4α-methylsterols, 5α(H):Δ^5 ratios are relatively low in C. hypolimnica and the sediments. A natural population of the dinoflagellate Peridinium lomnickii Woloszynska collected from the waters of Priest Pot (see Chapter 3) also contains the same suite of 4α-methylsterols and 5α(H)-stanols. The fact that all of the 4α-methylsterols recognised in P. lomnickii were present amongst the free lipids of the 0-6 cm sediment in similar relative amounts (Fig. 4.3/1) and the unique position of dinoflagellates among algae in synthesising 4α-methylsterols, have been used to postulate a dinoflagellate origin for the 4α-methylsterols present in Priest

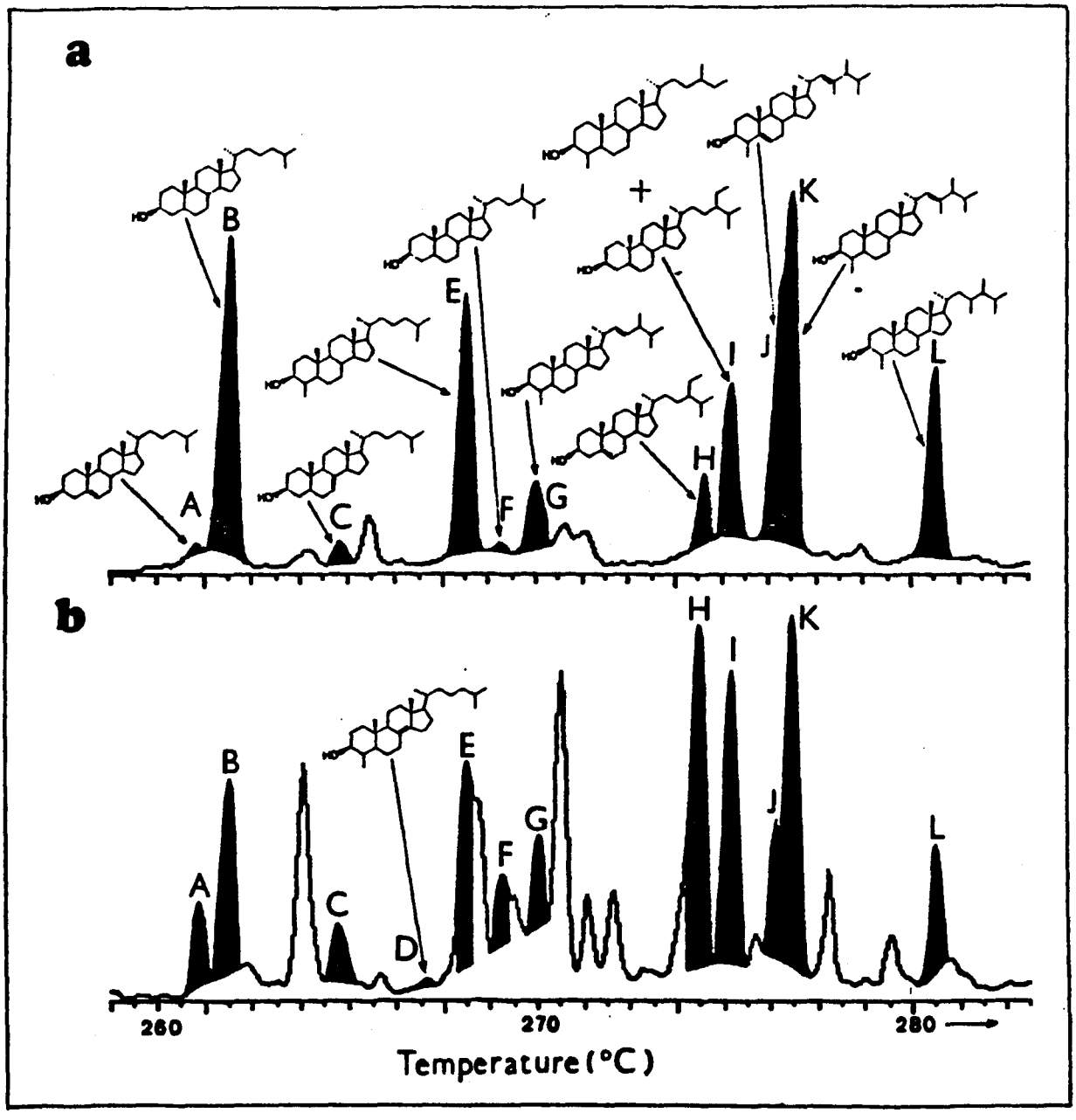


Fig. 4.3/1. Partial reconstituted ion chromatogram (RIC) from GC-MS analysis of the free alcohols (as TMS ethers) of:
(a) P. lomnickii Woloszynska;
(b) Priest Pot 0-6 cm sediment.
Sterol abundances are given in Tables 3.2/3 and 4.2/6. Instrumental conditions are given in Chapter 8. The sterols present in P. lomnickii [designated peaks in (a)] were all present in the sediment (b), together with various other sterols (mainly C_{27}-C_{29} Δ^5 and Δ^7 stenols and 5α(H)-stanols) derived from other sources (see text).

Pot and a dinoflagellate contribution to the sedimentary 5α(H)-stanols (Robinson et al., 1984a). One 4α-methylsterol, 4α-methyl-5α(H)-cholest-8(14)-en-3β-ol, was detected in the sediments of Priest Pot, but not in P. lomnickii, although it has been recognised in marine dinoflagellates and zooxanthellae (Kokke et al., 1981; Gagosian et al., 1980) and in freshwater P. cinctum (Chapter 3, this thesis). It may occur in P. lomnickii at growth stages other than that sampled, or in another species of dinoflagellate inhabiting Priest Pot. Alternatively, this $\Delta^{8(14)}$ sterol may reflect methanotrophic bacterial activity in the sediment, since it has been recognised in Methylococcus capsulatus (Bird et al., 1970; Bouvier et al., 1976).

Modification of the original 4-methylsterols is possible as 4α,23,24-trimethylcholesta-5,22-dien-3β-ol is not found in the rotifers, 4α-methyl-5α(H)-cholestan-3β-ol is found only in the C. hypolimnica and sedimentary lipids and 4α,24-dimethyl-5α(H)-cholest-22-en-3β-ol is only found in the sedimentary lipids. Among the organisms, the highest relative amount of 4α,23,24-trimethyl-5α(H)-cholestan-3β-ol is found in the C. hypolimnica sample. The organisms, however, were collected after the seasonal maximum abundance of dinoflagellates in Priest Pot, thus some of these differences may reflect the clearing of the dinoflagellate lipid signature from organisms high in the water column before the signature is lost from benthic organisms. Alternatively, the presence of another dinoflagellate species with a different sterol composition from P. lomnickii is possible, based on the absence of dehydrodinosterol in the rotifers.

Chlorophyceae, the dominant phytoplankton group in Priest

Pot, are known to display complex sterol compositions (Patterson, 1967, 1974; Nes and Mckean, 1977) including cholesterol and Δ^5, Δ^7, and $\Delta^{5,7}$ 24-methyl and 24-ethyl sterols. 24-Methyl and 24-ethyl $\Delta^{5,7,22}$ sterols (17 and 31 in Fig. 4.2/10) were detected in the rotifers and in lesser relative amounts in the ciliates; they were absent in all other fractions. 24-Methyl and 24-ethyl $\Delta^{5,7}$ sterols (28 and 39 in Fig. 4.2/10) were only detected in the rotifers. Thus it appears that, within the water column, $\Delta^{5,7}$ sterols from Chlorophyceae undergo a degradation which is complete on reaching the C. hypolimnica. The most likely mechanism is biochemical hydrogenation by organisms feeding upon the Chlorophyceae. In contrast, the Δ^7 sterols (30 and 40 in Fig. 4.2/10) do not suffer the same depletion, while cholest-7-en-3β-ol (13 in Fig. 4.2/10) was found only in the C. hypolimnica and free sedimentary lipids. $\Delta^{5,7}$ sterols have been found to be absent from the faecal pellets of a marine copepod fed on a diet of Chlorophyceae, with Δ^7 sterols being relatively enriched after passage through the gut of the copepod (Prahl et al., 1984). Biosynthesis of cholesterol, a Δ^5 sterol, involves the conversion $\Delta^7 \rightarrow \Delta^{5,7} \rightarrow \Delta^5$. Either the Δ^7 sterols are poorly assimilated during their passage through the gut, or else the organisms feeding upon the Chlorophyceae lack the enzymes necessary to effect the $\Delta^7 \rightarrow \Delta^{5,7}$ conversion and cannot make use of these sterols present in their diet.

24-Methyl- and 24-ethylcholest-5,7,9(11),22-tetraen-3β-ol sterols were tentatively identified in the rotifer and ciliate lipids from Priest Pot. Preservation of these labile molecules in the sediments was not expected. There have been no reports of

reports of such sterols in algae. Itoh et al. (1983) proposed that $\Delta^{5,7,9(11),22}$ sterols found in a sponge arose through biochemical dehydrogenation of dietary sterols. Certain protozoa such as Tetrahymena pyriformis are known to be able to introduce double bonds into sterols (Nes and McKean, 1977). Alternatively these sterols may be formed by de novo synthesis.

The hydrogenation of Δ^5 sterols to 5α(H)- and 5β(H)-stanols by a bacterial mechanism is believed to occur in lake sediments (Ogura and Hanya, 1973; Gaskell and Eglinton, 1976). The mechanism is proposed to proceed in the following manner:

$$\Delta^5\text{-sterol} \rightarrow \Delta^4\text{-3-one} \begin{cases} \rightarrow 5\alpha(H)\text{-stanone} \rightleftarrows 5\alpha(H)\text{-stanol} \\ \rightarrow 5\beta(H)\text{-stanone} \rightleftarrows 5\beta(H)\text{-stanol} \end{cases}$$

The interconversion of stanols and stanones in sediments was demonstrated by the incubation of 4-^{14}C-5α(H)-cholestan-3β-ol in an algal mat, in which 5α(H)-cholestan-3-one was one of the products (Edmunds et al., 1980). It is interesting, therefore, that going down the water column, a change towards higher amounts of 5β(H)-stanols and higher 5α(H):Δ^5 ratios is first apparent in the bacterial C. hypolimnica sample, with even higher values in the free sedimentary lipids and that this is accompanied by the first detectable amounts of stanones (Table 4.2/7; Fig. 4.3/2). 3α-OH stanols, only detected in the C. hypolimnica and the sedimentary lipids, have never been found in any organism, but formation of stanones followed by reduction to stanols would allow for epimerisation of the 3-position to give the thermodynamically more stable 3α-OH isomer. The bound sterols showed a lower 5α(H):Δ^5 ratio and a higher relative amount of C$_{27}$

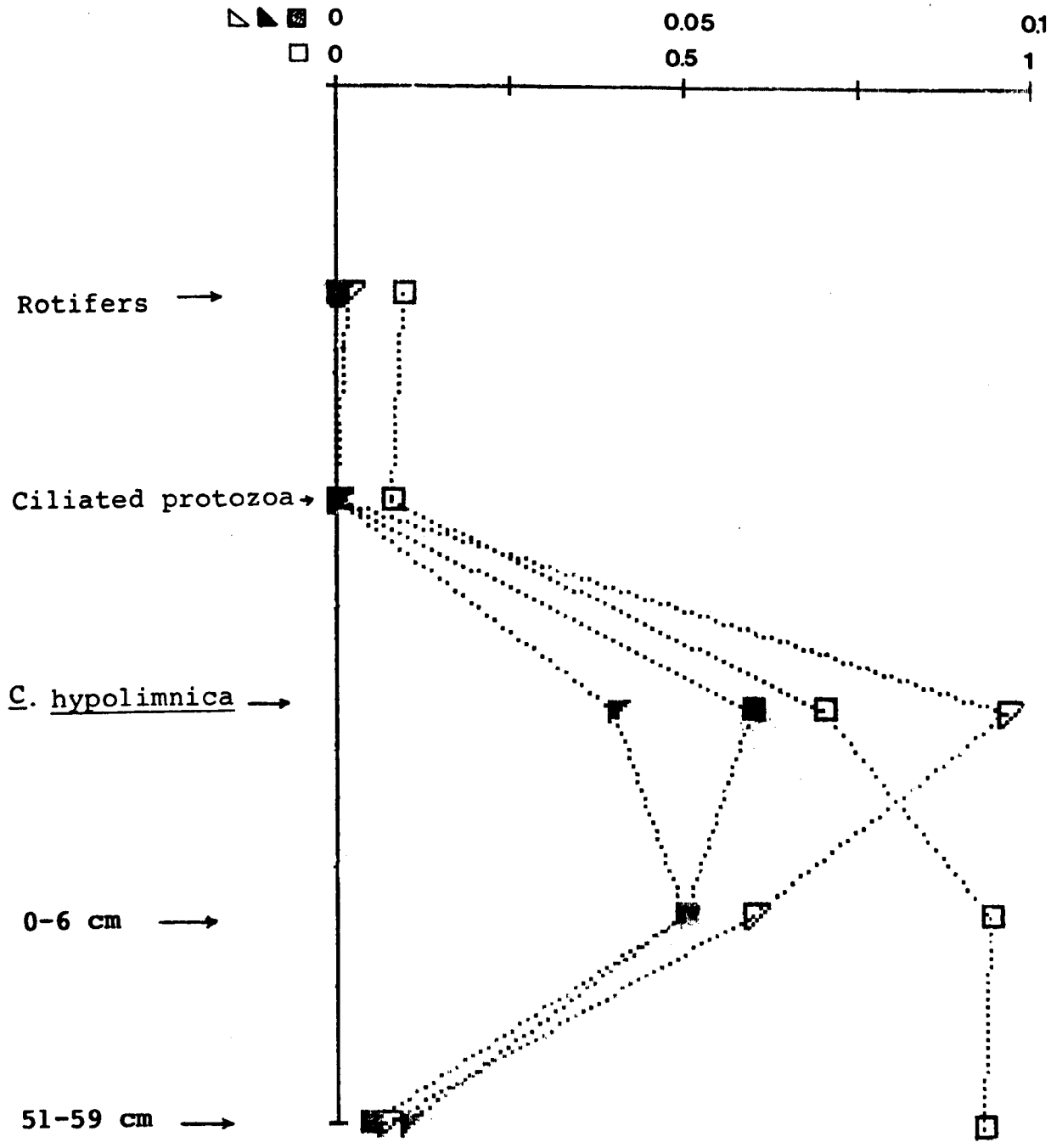


Fig. 4.3/2. Depth profile of various lipid parameters through Priest Pot water column and sediments showing proposed bacterial influence on sterol transformations in the lacustrine environment.

□ 5α(H):Δ^5 ratio, obtained by summing individual abundances of cholestanol-cholestanol and 24-methyl and 24-ethyl analogues.

◺ 5β(H) STANOLS/TOTAL STEROLS

◣ 3α-OH/TOTAL STEROLS

■ STEROIDAL KETONES/STEROLS

sterols than the free lipids, consistent with greater stability to diagenetic change and a larger microbial input for the bound sterols.

4α-Methylsterols are relatively more abundant in the free lipids of the deeper sediment than in the surface sample. Thus the presence of the 4α-methyl group appears to confer greater resistance to degradation, although the increase in abundance of 4α,24-dimethyl-5α(H)-cholestan-3β-ol and of 4α,23,24-trimethyl-5α(H)-cholestan-3β-ol with respect to their Δ^{22} unsaturated analogues, suggests that hydrogenation of the Δ^{22} double bond of 4α-methylsterols does take place within the sediment. 4-Methylsterols have been proposed to be more resistant to degradation than desmethylsterols in the marine environment (Gagosian et al., 1980). Among the desmethylsterols C_{27} components appear to be transformed more rapidly than C_{29} sterols, a phenomenon that has also been observed in microbial mats (Edmunds, 1982; Boudou et al., 1984).

The low abundance of Δ^7 sterols in the 51-59 cm sediment (Table 4.2/6), presumably reflects a smaller input from Chlorophyceae to that sediment layer than to the surface sediments. Dinoflagellates, however, appear to have been at least as prominent amongst the phytoplankton of the lake, at the time of deposition of the 51-59 cm sediment section, as they are today.

4.3.iii Ketones

Present evidence suggests that there are two major sources for the steroidal ketones found in sediments: formation by microbial degradation of sterols, as demonstrated by radiolabelling studies (Edmunds et al., 1980); and contribution as direct biolipid inputs from planktonic or other organisms, despite the sparsity of such reported occurrences (Gagosian and Smith, 1979). Dinosterone has been identified in a cultured marine dinoflagellate (Withers et al., 1978) and in marine sediments (Gagosian and Smith, 1979; Brassell et al., 1981; Smith et al., 1983) where, like dinosterol, it has been taken as an indicator of dinoflagellate input. Other 4α-methylstanones have not been detected in marine organisms. Such compounds and dinosterone occur, however, as major components of the free ketone fraction isolated from a natural population of P. lomnickii collected in Priest Pot. The proposed role of dinoflagellates as contributors of 4α-methylsteroids to sediments is supported by the occurrence, in the underlying bottom sediment of Priest Pot (Fig. 4.3/3B), of all of the 4α-methylstanones recognised in P. lomnickii (Fig. 4.3/ A), together with various 4-desmethylsterones derived from other organisms or microbial oxidation of sterols.

C_{27}-C_{29} Steroidal ketones having a 3,5-dien-7-one structure are commonly found in the bound lipids of lacustrine sediments

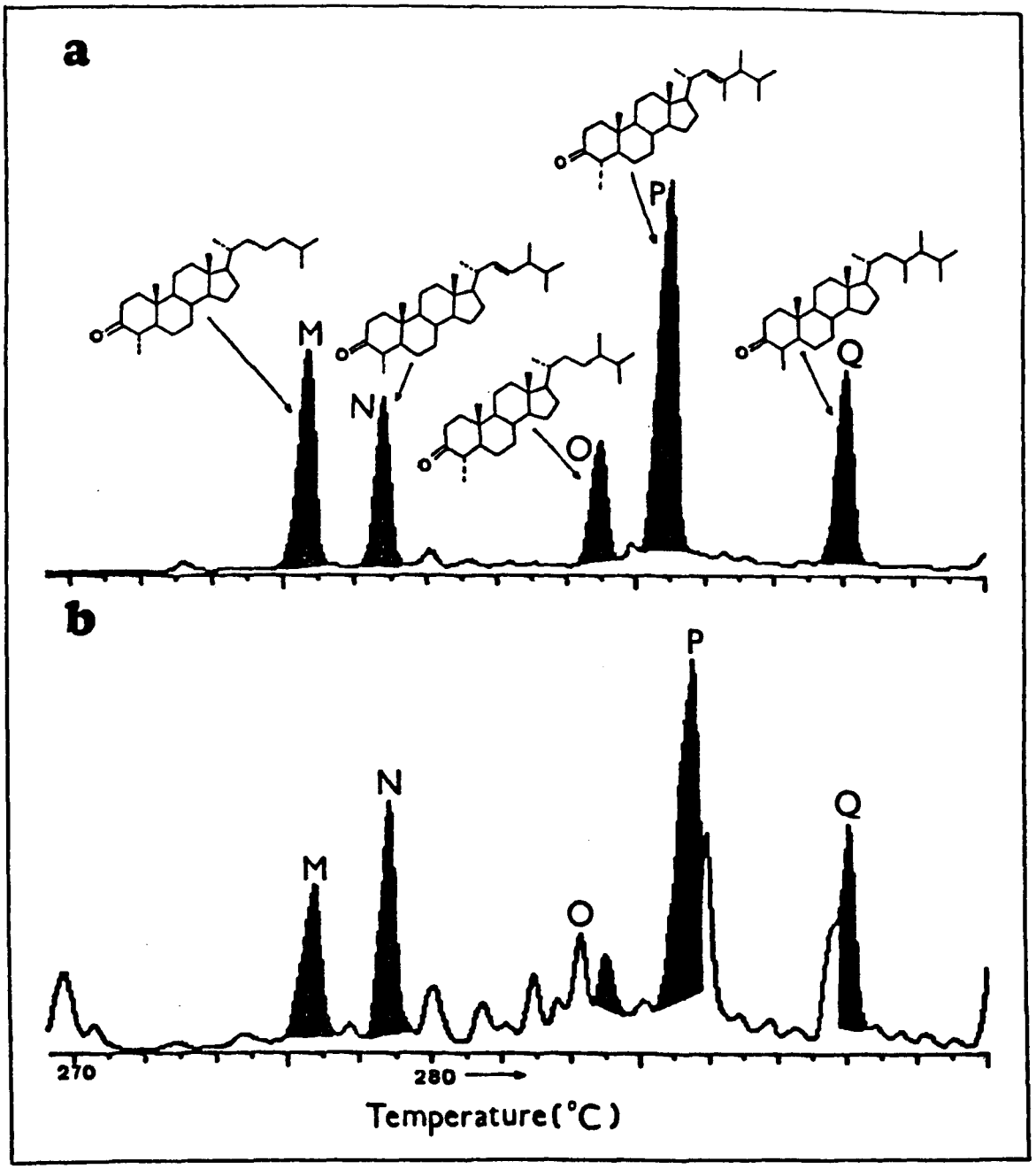


Fig. 4.3/3. Partial RIC from GC-MS analysis of the free ketone fraction of: (a) P. lomnickii; (b) Priest Pot 0-6 cm sediment. Stanone abundances are given in Tables 3.2/2 and 4.2/7. Instrumental conditions are given in Chapter 8. The 4α-methylstanones present in P. lomnickii [designated peaks in (a)] were all recognised in the sediment (b), together with 5β(H)- and 5α(H)-stanones derived from other sources or formed by in situ microbial oxidation of sterols (see text).

with relative abundances in the same ratio as the corresponding sterols. Their origin is unknown, but the corresponding dienol has been suggested as a microbially-formed intermediate in the formation of steratrienes (Gagosian and Farrington, 1978).

A homologous series of alkan-2-ones frequently occurs in the lipids of lacustrine (Cranwell, 1977, 1981) and intertidal marine (Volkman *et al.*, 1983) Recent sediments. Such compounds are not primary plant products (Tulloch, 1976). A derivation from *n*-alkanes by microbiological oxidation has been postulated (Allen *et al.*, 1971), which may take place before deposition in the aquatic environment (Cranwell, 1981a). The general similarity of the *n*-alkane and alkan-2-one distributions in Priest Pot sediments (*cf.* Figs. 4.2/3 and 4.2/11) and the increase in abundance of bound alkan-2-ones in the deeper sediment section, provide evidence for an *in situ* microbiological origin of the sedimentary alkan-2-ones. Furthermore, a series of the proposed alkan-2-ol intermediates occurs in the free lipids of the 51-59 cm sediment, with an almost identical distribution to the free alkan-2-ones of the same sample (Fig. 4.3/4). Such a relationship has also been recognised in an oil shale (Chicarelli *et al.*, 1984). The bound alkan-2-ones of the 51-59 cm section resemble the free alkanes of the same sample, rather than the bound alkanes. Together with the absence of bound alkan-2-ones in the 0-6 cm sediment and the proposed *in situ* formation of free alkan-2-ones, this suggests that in Priest Pot bound alkan-2-ones arise by binding of free alkan-2-ones, rather than oxidation of bound alkanes.

The isoprenoid 6,10,14-trimethylpentadecan-2-one occurs

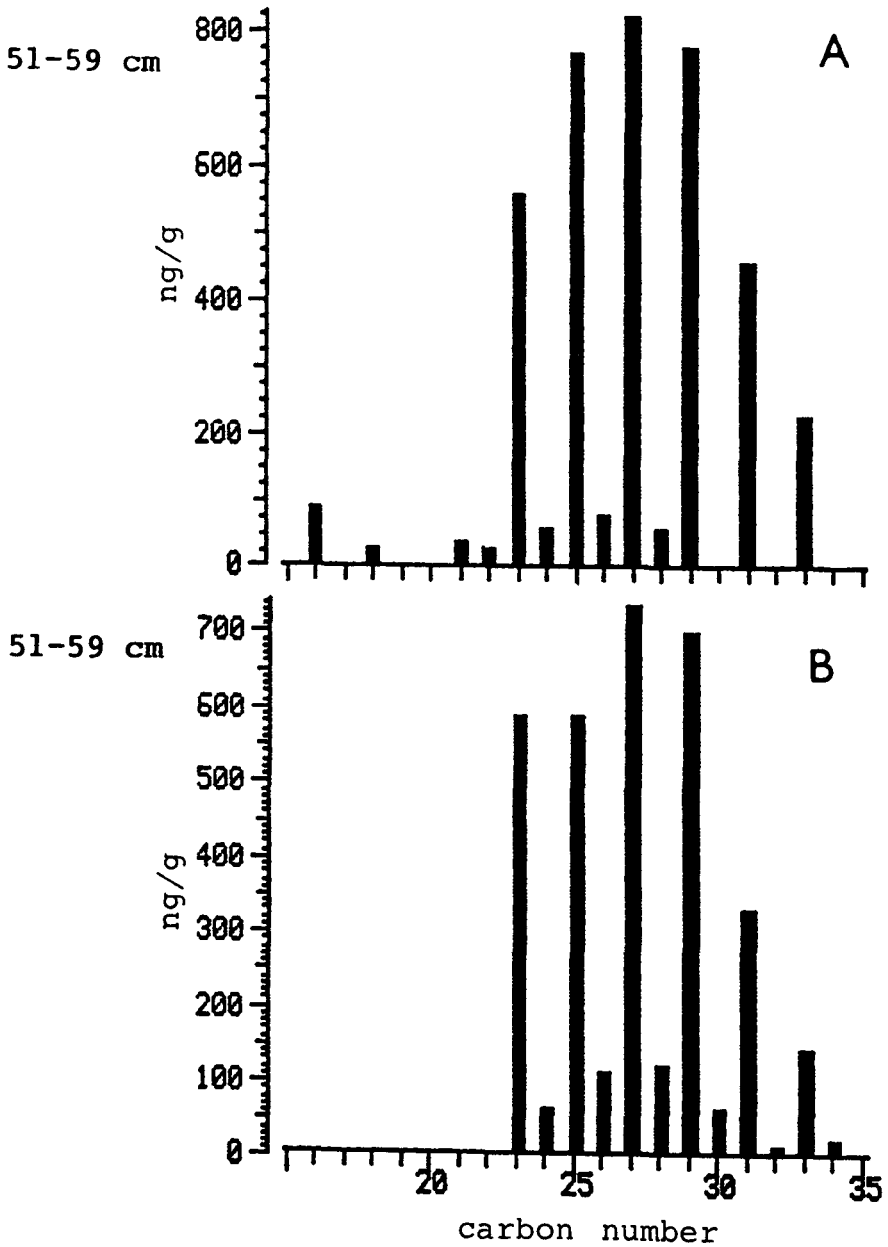


Fig. 4.3/4. Chain length distributions of: (A) alkan-2-ols isolated from the free lipids of the 51-59 cm sediment section; (B) alkan-2-ones isolated from the free lipids of the 51-59 cm sediment.

Quantitation expressed as ng g dry, extracted sediment^{-1}, determined by: (A) comparison of GC peak areas with that of a known amount of $\underline{n}$-C_{18} alkanol, all as TMS ethers;

(B) comparison of GC peak areas with those of known amounts of $\underline{n}$-C_{18} and $\underline{n}$-C_{28} alkanes.

widely in sediments and has a stereochemistry compatible with an origin from phytol (Brooks et al., 1978). The differences in the triterpenoid ketone distributions of the two sediment sections suggests a difference in higher plant input and may reflect changes in the marginal vegetation of Priest Pot.

4.3.iv Hydrocarbons

n-Heptadecane occurs as the major n-alkane in many algae (Han et al., 1968; Han and Calvin, 1969; Gelpi et al., 1970; Blumer et al., 1970) and may also originate from certain bacteria (Cranwell, 1976a,b). Hence, the alkane content of the ciliated protozoa and C. hypolimnica samples presumably reflects the algal diet of these organisms. The low amounts of n-alkanes present in the rotifer sample raises the possibility that the distribution observed has been affected by contamination, either in the natural environment or during handling; however, distributions of n-alkanes showing no odd-even predominance over a wide molecular weight range are said to occur in certain bacteria (Davis, 1968; Han and Calvin, 1969), although confirmation is desirable.

The distribution of the free n-alkanes isolated from the 0-6 cm sediment shows a weak maximum at C_{17} and a high proportion of C_{27}, C_{29} and C_{31} components (Fig. 4.2/5), suggesting the majority of the sedimentary n-alkanes originate from higher plants, as such distributions are characteristic of the alkane content of higher plant epicuticular wax (Eglinton and Hamilton, 1967; Cranwell, 1973). n-Alkanes in the 51-59 cm section also originate

largely from higher plants, but the distribution is quite different to the surficial sediments, containing a larger C_{23}, C_{25} and C_{27} contribution and relatively less n-C_{31}. Although the abundance of free n-alkanes decreases with increasing sediment depth, diagenesis is unlikely to have caused this difference in distribution as it would be expected to preferentially remove n-C_{23} relative to n-C_{31} (Quirk, 1978; Cranwell, 1981a). A weak maximum at C_{23} often occurs in sedimentary n-alkane distributions (Cranwell, 1982) and it was dominant in the sediment and overlying algal mat of Laguna Lejia (Simoneit et al., 1980). n-C_{23} has been reported as the dominant alkane of fresh Sphagnum by Quirk (1978) but, as decay occurred, the alkane distribution became dominated by C_{31}, as in the underlying peat profile. The differences in distribution between the two sediment sections may, therefore, reflect differences in source organisms, possibly associated with the change in tree cover around the lake, or a shorter period of decomposition of allochthonous material before deposition in Priest Pot for the deeper sediment.

Cranwell (1981a) has shown that, in Recent sediments from the oligotrophic Loch Clair, the biodegradability of free lipids increase in the order n-alkanes, alkan-2-ones, sterols, n-alkanoic acids, n-alkanols, n-alkenoic acids. In Priest Pot sediments the relative decrease of free n-alkanes with depth is much greater than for free n-alkanols (cf. Figs. 4.2/3 and 4.2/7). Bound n-alkanes, however, are ca. 10x more abundant in the 51-59 cm section than in the surface sediments and are presumably formed by conversion of free to bound lipids during diagenesis in the sediment. If there is a chain length dependence of such a

conversion this should be reflected in the distribution of residual free alkanes. Bound n-alkanes having a similar distribution to those of the 0-6 cm section have been reported to occur in detritus resulting from aerobic decay of cyanobacteria (Cranwell, 1979) and in surface sediments of Loch Clair (Cranwell,1981a), where they were attributed to a bacterial origin. Preferential degradation of shorter chain homologues in Loch Clair resulted in a bound alkane distribution in which C_{29}, C_{31} and C_{33} alkanes were prominent (Cranwell, 1981a). A similar process may be occurring in Priest Pot sediments; alternatively, the bacterial contribution to the 51-59 cm section bound hydrocarbons may have been obscured by a large scale conversion of higher plant-derived free alkanes to a bound form.

Isobotryococcene (XLIX) has been identified in the colonial green alga Botryococcus braunii (Cox et al., 1973), but botryococcene (L) was the dominant hydrocarbon. The presence of isobotryococcene in the ciliated protozoa presumably reflects the algal diet. Small populations of B. braunii have been observed in the waters of Priest Pot. The absence of botryococcene may be due to the existence of strains of B. braunii which produce different proportions of isobotryococcene, physiological factors or biochemical modification of botryococcene by the ciliates; alternatively, there may be other algae producing isobotryococcene in Priest Pot, although a culture of the major colonial green alga, Dictyosphaerium sp., related to B. braunii, does not produce isobotryococcene or botryococcene (Dr. P.A.Cranwell, personal communication). The absence of isobotryococcene in the rotifer sample may reflect the inability

of these organisms to feed on colonial algae, as rotifers are smaller than Loxodes magnus and striatus. Isobotryococcene was not detected in the sediments, implying that either it is not preserved, or else its relative abundance in the ciliated protozoa sample is caused by biological concentration.

n-Alkenes in lacustrine sediments were first reported from Greifensee, a productive lake, in which odd-carbon homologues were major components, and were attributed to higher plant input because similar olefins were detected in the leaves of reeds (Giger and Schaffner, 1977). Cranwell (1981a, 1982) reported free n-alk-1-enes in the C_{19}-C_{33} range, with even carbon number predominance maximising at C_{26}, in sediment from the oligotrophic Loch Clair. Distributions similar to those in Loch Clair were observed in a peat profile and in Sphagnum and Calluna spp. (Quirk, 1978) and homologous alk-1-enes with prevalent even homologues in leaf waxes (Stransky et al., 1970). Free alkenes in the C_{18}-C_{32} range, maximising at C_{29} or C_{31}, however, were reported to occur in peat and the fern Dryopteris dilatata collected from the vicinity of Rostherne Mere (Cardoso et al., 1983). The surficial sediments of Rostherne Mere contained a similar homologous series of alkenes with odd over even predominance, maximising at C_{25}. Priest Pot sediment contained free alk-1-enes in the C_{16}-C_{27} range, with odd-carbon number predominance maximising at C_{25} (Fig.4.2/6). As in the case of Greifensee (Giger and Schaffner, 1977) the origin of the alk-1-enes in Priest Pot sediments may be reeds, as these are prevalent in the margins of the lake, although some other source such as bacteria cannot be ruled out. The observed decrease in

relative abundance of alkenes with increasing sediment depth in Priest Pot is consistent with the greater lability of unsaturated compounds (Kawamura et al., 1980; Cranwell, 1981a; Cardoso et al., 1983). The presence in the sediment of phytenes and phytadienes presumably reflects phytol diagenesis.

Hopanoids occur widely in sediments and are accepted as originating largely from bacteria (e.g. Ourisson et al., 1979). Hop-22(29)-ene, dominant in Priest Pot sediments, is often the major hopene of contemporary sediments (Brooks, 1974) and represents direct bacterial input. Isomerisation of hop-22(29)-ene to hop-21-ene and thence to hop-17(21)-ene occurs in sediments (Ensminger, 1977). This isomerisation has proceeded further in the older 51-59 cm sediment section (Table 4.2/4). Inwash of fragments of mature rocks eroded from the catchment area may have given rise to the small amounts of 17β(H),21α(H)- and 17α(H),21β(H)-hopanes present in Priest Pot, as these compounds are produced by diagenetic epimerisation of the naturally occurring 17β(H),21β(H)-hopanes; alternatively, such compounds may reflect anthropogenic fossil fuel burning activities. 22R-17α(H),21β(H)-Homohopane, the most abundant 17α(H),21β(H)-hopane in Priest Pot, has been detected in a peat (Taylor et al., 1980) and thus may have a direct biological origin. 17β(H),21β(H)-hopanes are relatively more abundant in the older 51-59 cm section than in the surface sediments. In the 51-59 cm layer some 17β(H),21β(H)-hopanes may have arisen from diagenesis of bacterial products such as polyhydroxybacteriohopanes; similarly the sedimentary hopanoid alcohols, ketones and acids are probably early-stage diagenetic

products of polyhydroxybacteriohopanes (cf. Rohmer et al., 1980b). The possible input of hopanes having an anthropogenic origin would presumably have been smaller to the 51-59 cm sediments than to the surficial sediments,

Δ^2 Sterenes were found in the C. hypolimnica sample and the free sedimentary lipids, with $\Delta^{3,5}$ steradienes and steratrienes also present in the free sedimentary lipids (Table 4.2/3). These steroidal hydrocarbons have not been observed or are present only in trace amounts in oxic marine or lake sediments, whereas they are abundant in anoxic marine sediments(Gagosian et al., 1980; Quirk et al., 1980). $\Delta^{3,5}$ Steradienes are believed to originate from direct dehydration of Δ^5 sterols, with Δ^2 sterenes arising from dehydration of 5α(H)-stanols. These transformations may be due either to a clay-catalysed chemical process or a microbial process. The high degree of side-chain selectivity observed suggests a microbial involvement (Maxwell and Wardroper, 1982), and this appears to be the case in Priest Pot, as the carbon number distribution of the steroidal hydrocarbons is different from that of the sterols. In common with most other contemporary sediments 4-methylsterenes were not present.

C_{27}-C_{29} Steryl chlorides were present in the bound hydrocarbon fraction isolated from the 51-59 cm section, having the same relative abundances as the corresponding sterols. Formation presumably took place by an S_N1 mechanism during reflux of the pre-extracted sediment with 6N HCl (Sykes, 1961). Elimination of a proton from the carbonium ion intermediate produced in such a reaction would also have produced the $\Delta^{3,5}$ steradienes and $\Delta^{3,5,22}$ steratrienes present in the same

fraction. Acid treatment of 24-ethylcholesta-3,5-diene is known to produce 4-methyl,24-ethyl-19-norcholesta-1,3,5(10)-triene and B ring anthrasteroids (Hoffmann, 1984). Although 4-methyl,24-ethyl- 19-norcholesta-1,3,5(10)-triene and B ring anthrasteroids occur in sediments (Hussler and Albrecht, 1981; Hoffmann, 1984) they have never been observed in such a young or shallow buried sediment as Priest Pot, their presence in Priest Pot is, therefore, taken to result from the extraction procedure employed.

4.3.v Acids

n-Alkanoic acids with chain lengths greater than 20 have been attributed to input derived predominantly from higher plants while those in the C_{12}-C_{18} range have been attributed to aquatic organisms (Simoneit, 1978). The organisms and surface free n-alkanoic acids of Priest Pot are dominated by C_{12}- C_{18} constituents with C_{16} maximal, consistent with the high productivity of Priest Pot. The bound acids of the 0-6 cm sediment contained a greater relative abundance of n-C_{16} and iso- and anteiso-branched acids, especially ai-C_{15}, reflecting the larger bacterial contribution to bound lipids, previously observed in other sediments and algal detritus (Brooks et al., 1976, 1977; Cranwell, 1978, 1979). Free n-alkanoic acids have decreased in abundance in the deeper sediment section, while bound acids were more abundant than in the surficial sediments.

The free acids of the 51-59 cm sediment show a slight decrease in relative abundance of shorter chain homologues; similarly, $< C_{20}$ acids are relatively more abundant in the bound lipids of the deeper section.

Alkenoic acids were present in the organisms, probably biosynthesised by the organisms themselves. Diagenetic removal of these labile compounds with increasing time is apparent and appears to proceed within the water column, the polyunsaturated acids present in the ciliated protozoa being absent from the underlying C. hypolimnica sample (Table 4.2/9). The relatively greater abundance of alkenoic acids amongst the bound sedimentary lipids than the free reflects the stabilisation afforded to labile lipids present in a bound form (Cranwell, 1981a).

Similar distributions of α,ω-dicarboxylic acids to those present in Priest Pot sediments have been reported to occur in other lacustrine sediments (Cranwell, 1977; Ishiwatari et al., 1980). A number of possible origins have been proposed (Cranwell, 1982 and references therein), but the similarity of the distributions of α,ω-dicarboxylic acids and ω-hydroxy acids and the increase in abundance with increasing sediment depth, suggest that, in Priest Pot, microbial oxidation of ω-hydroxy acids occurs (cf. Cardoso and Eglinton, 1983). ω-Hydroxy acids are present in both microorganisms and higher plants, where they may exist in a free form in cuticular waxes or in a bound form in the polymers cutin (C_{16},C_{18}) and suberin (range C_{16}-C_{24}) (e.g. Holloway, 1973). They may also be formed by microbial transformations of other lipid classes, such as alkanoic acids (Boon et al., 1977). Even-carbon number predominant series of

free and bound w-hydroxy acids, maximising at C_{16} and C_{22}, have been reported for surface sediments of Rostherne Mere, a productive lake, and for a 5000 year old lacustrine sediment from Esthwaite Water (Cardoso et al., 1977; Cardoso and Eglinton, 1983) and for surface sediments of Coniston Water (Chapter 2, this thesis). In Priest Pot sediments, bound w-hydroxy acids are far more abundant than free compounds. The increase in abundance of w-hydroxy acids with increasing sediment depth (Fig. 4.2/14B) implies that there has been a change in input, or, more likely, in situ formation of bound w-hydroxy acids.

Bound 3-hydroxy acids with a similar distribution to that observed in Priest Pot sediments (Fig. 4.2/14A) occur widely in recent sediments (Cardoso and Eglinton, 1983) and have been shown to originate from bacterial lipopolysaccharides (Cranwell, 1981b; Klok, 1984). The good preservation of bound 3-hydroxy acids in the 51-59 cm sediment section, in spite of the readily metabolisable nature of such compounds (Boon et al., 1977), is further evidence of the stability imparted to bound acids. The bound 2-hydroxy acids detected in the 0-6 cm sediment sample probably originate from higher plant sources, in which they are by-products of the α-oxidation of fatty acids (Cranwell, 1981b).

4.3.vi Wax esters

Higher plants possess even-carbon number straight chain saturated esters in the C_{32}-C_{64} range (Tulloch, 1976). C_{32}-C_{52}

Alkyl esters, maximising in abundance at C_{44}, have been isolated from recent lacustrine sediments (Cranwell and Volkman, 1981), where they were attributed to higher plant input. The distribution of alkyl esters in Priest Pot sediments (Table 4.2/9) is also consistent with a major allochthonous input of wax esters, although the C_{28}-C_{33} straight chain and iso-/anteiso-branched components may originate from bacterial sources (Cranwell, 1983). Differences in molecular composition of C_{42} to C_{46} wax esters between the two sediment layers may reflect changes in the marginal vegetation of the lake. The steryl esters detected in Priest Pot sediments may originate from higher plant or aquatic source organisms. Phytyl octadecanoate, detected amongst the wax esters isolated from the 51-59 cm sediment, also occurs in Peridinium cinctum (Chapter 3, this thesis).

4.3.vii Aldehydes

Aldehydes occur as components of higher plant epicuticular waxes (Kolattukudy, 1976). A homologous series of straight chain aldehydes isolated from a lacustrine Brazilian oil shale, and having a similar distribution to the alkanes of the same sample, were proposed to originate from microbial oxidation of the alkanes (Chicarelli and Cardoso, 1983). The aldehydes present in the free lipids isolated from the 0-6 cm sediment of Priest Pot, including polyunsaturated compounds, probably originate from a direct input. Such compounds would be expected to be rapidly

degraded in the aquatic environment, presumably by oxidation, and, indeed, they were absent from the 51-59 cm section.

4.4 Conclusions

The fate of lipids in a small, productive lake has been investigated by an approach involving the analysis of natural populations of organisms from different depths in the water column, and analysis of two sections of a short core of the underlying bottom sediments. Aquatic microorganisms were demonstrated to be sources of sedimentary lipids and key agents modifying the original biolipid distributions, with transformations occurring both within the water column and in the sediment. Although more information on feeding pathways and turnover times is desirable, several preliminary conclusions can be drawn concerning the fate of biolipids in Priest Pot.

1) The lipids of the rotifers and ciliated protozoa reflect the algae which they feed upon. Thus, $\Delta^{5,7}$ and Δ^{7} sterols, derived from the population of Chlorophyceae in the lake, are constituents of lipid extracts of the rotifer and ciliated protozoa samples; isobotryococcene was present in the ciliated protozoa, probably originating from feeding of these organisms on algae such as B. braunii; the rotifers did not contain isobotryococcene, possibly because the size of B. braunii, or other source organism, precludes the rotifers from feeding upon it; 4α-methylsterols were present in both the rotifers and ciliated protozoa, occurring as a result of feeding by these

organisms on the population of the dinoflagellate P. lomnickii in Priest Pot. Sterols, including the 4α-methylsterols, were constituents of the free lipid extract of the C. hypolimnica sample, but, as for the rotifers and ciliated protozoa, originate from algae.

2) 4α-Methylsterols, present in the sediment, were assigned to an origin from P. lomnickii, based on their similarity of distribution in the sediment and the dinoflagellate. A similar dinoflagellate origin is proposed for the sedimentary 4α-methylsteroidal ketones and for some of the sedimentary 5α(H)-stanols.

3) Δ^7 Sterols, derived from the Chlorophyceae, are resistant to degradation and are preserved in the sediments.

4) An input of C. hypolimnica lipids to the surface sediments was demonstrated by the isolation of farnesol from both samples.

5) Aquatic source organisms provide an input of mainly C_{17} to the sedimentary n-alkanes, but this is minor compared with the allochthonous input of hydrocarbons.

6) Hexadecan-2-ol is present in the rotifer, ciliated protozoa and C. hypolimnica samples and in the sedimentary free and bound lipids, but with differing enantiomeric compositions.

7) Microbial modification of the original biolipid distributions occurs; such modification commences within the water column during sedimentation from the photic zone by zooplankton feeding processes and bacterial attack, and continues after incorporation into the sediments. $\Delta^{5,7}$ Sterols are rapidly removed within the water column and do not reach the sediments. Bacterial hydrogenation of Δ^5 sterols via ketone intermediates is

supported by contemporaneous increases in 5β(H)-stanols and 5α(H):Δ^5 ratios and by the formation of stanones and 3α-OH stanols in the bacterial C. hypolimnica layer, with further increases observed for the sediments.

8) Straight chain alkanols, alkanes and alkanoic acids show a preferential degradation of shorter chain homologues.

9) Unsaturated molecules are degraded more rapidly than saturated compounds, although unsaturated lipids are better preserved in Priest Pot sediments than in the sediments of the oligo-mesotrophic Coniston Water (see Chapter 2).

10) 4α-Methylsterols are better preserved than desmethylsterols.

11) A microbiological oxidation of alkanes to produce the sedimentary alkan-2-ones is suggested by their observed similarities of distribution, the increase in abundance with increasing sediment depth and the presence of a similar chain length distribution of the proposed alkan-2-ol intermediates in the 51-59 cm sediment section.

12) Formation of Δ^2 sterenes and $\Delta^{3,5}$ steradienes occurs, most likely by a bacterial process. The presence of such compounds, together with rearrangement products, amongst the bound lipids of the 51-59 cm sediment, is proposed to be an artifact of the extraction procedure.

13) Bound lipids show greater preservation and a higher input of microbial lipids than do the free lipids. Conversion of free to bound lipids apparently takes place during the early stages of lipid diagenesis for certain compound classes.

Selective biomass sampling, as in this work, provides a useful means for the study of sedimentation in a water column.

CHAPTER FIVE

LAKE KINNERET

5.1 INTRODUCTION

The ice cover which extended over northern Europe and America in the Pleistocene, did not, in general, allow the survival of Tertiary water bodies and their fauna. Hence, most of the lakes of the world are not older than 20000 years. A few exceptions to this general pattern exist; lakes Baikal, Tanganika, Ohrid and the Caspian Sea, various lakes in the Celebes, in China and Lake Kinneret (Sea of Galilee) in Israel can be considered as relict lakes from the Tertiary era. Lake Kinneret occupies a special position among the relict lakes of the world, acquiring its present configuration just 20 000 years ago. Previously, however, during the Neogene and Pleistocene, inland water bodies existed in the developing Jordan Valley. A number of species were preserved in these environments and populated the lakes of the Jordan Valley as they developed. Thus a relict fauna exists in Lake Kinneret and, also, the long period of isolation has led to a certain endemism. A relict fauna and endemism are two important characteristics shared in common by all relict lakes. The high degree of tectonic activity that has taken place in the Jordan Valley and the resultant variations of water level and salinity, did not allow the development of reliction and endemism to such a high degree as found in Lake Baikal and Lake Ohrid for example (Serruya, 1978).

Although the geology, hydrology, biology and general chemistry have been described in some detail (Serruya, 1978), there is no published data on the organic geochemistry of Lake Kinneret. The unique position of Lake Kinneret amongst the lakes of the world makes an investigation of its organic geochemistry

worthwile for this reason alone, but the lake acquires a special significance due to the dominance of the phytoplankton by a single species of dinoflagellate, Peridinium cinctum fa. westii (Lemm.) Lef., which may account for up to 88% of the total biomass. Much of the following description of the biology, geography and chemistry of Lake Kinneret and the surrounding areas is taken from Serruya (1978). In general, regular sequences of algal succesion are seen in Lake Kinneret; there is evidence to suggest that blooms of P. cinctum have been a regular feature in Lake Kinneret since at least 1893, regular records have been kept since 1968. After an exponential phase of growth (November-late March), the Peridinium population remains stable forming 99% of the total phytoplankton biomass until late April and then declines, but still dominates until May-June. In summer and autumn, following the Peridinium bloom, Chlorophyta and Cyanophyta form the bulk of the algal biomass. P. cinctum has been described in detail by Pollingher (1978); its lipid content is reported in Chapter 3 of this thesis.

The biomass of zooplankton in Lake Kinneret ranges from 24 to 56 g m^2 wet weight. The average contribution of the different groups, based on the results of seven years, is as follows: Cladocera 58%, Copepoda 35% and Rotifera 7%. Few zooplankton have been observed to graze P. cinctum, possibly because of the latter's large cell size and the presence of a theca, one exception is the uncommon rotifer Asplanchna. Dense populations (10^7 cells ml^{-1}) of the photosynthetic sulphur bacterium Chlorobium phaeobacteroides develop in the anoxic, sulphide-containing metalimnion and form a red-brown layer from July to September almost every year.

Lake Kinneret is situated in the central part of the Jordan Rift Valley and has a catchment area of 2730 km^2 at altitudes ranging from 2814 m to -210 m (Fig. 5.1/1). Such a large range of altitudes produces a variety of landscapes and vegetation. Three main vegetation belts exist in the Hermon Mountain: (1) up to 1400 m, oak forest dominates; (2) from 1400 to 1800 m, the destructive influence of Man has reduced the arborescent vegetation to sparse dwarf trees not exceeding 1 m in height, most of the area is covered by thorny plants; (3) above 1800 m, a type of Alpine vegetation develops, consisting of thorny plants adapted to the dry summer conditions. The Golan Heights in the east are covered with basalt, many of the slopes are barren, although oak forests are found in two areas. The irrigated areas of the Hula plain support vegetable, flower and fruit production. In the immediate surroundings of the lake, vegetation is of the semi-arid type; along the lake shore Abraham's bush, oleander, reeds, bullrushes and raspberry bushes occur.

Lake Kinneret has a surface area of 167.8 km^2 and a maximum depth of 43 m with a mean depth of 25.6 m. The bathymetric curves of the lake have the general form of an eccentric ellipse, being closely packed near the eastern shore. The deepest part of the lake is located slightly NE of the centre and approximately 8 km south of the mouth of the Jordan. Lake Kinneret is normally stratified from mid-March to mid-December, the thermocline dividing the lake into two almost independent water masses: a warm, oxygenated, CO_2-depleted epilimnion with an abundant algal flora and a cold, oxygen-depleted hypolimnion with dissolved H_2S and CO_2. Complete turnover down to 42 m generally occurs in late

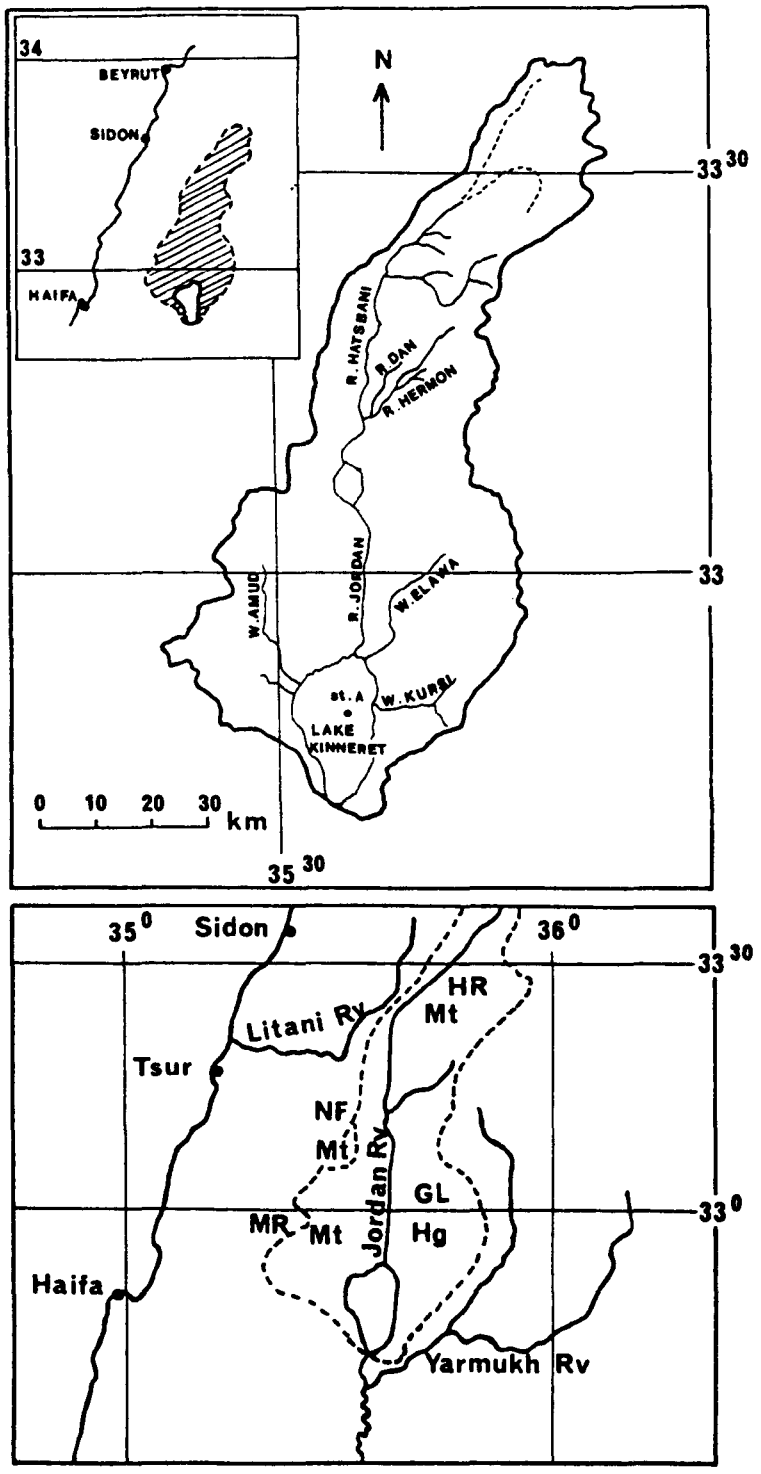


Fig. 5.1/1. Lake Kinneret and its watershed. HR Mt = Hermon Mountain, Nf Mt = Naftali Mountain, MR Mt = Meron Mountain, GL Hg = Golan Heights.
(Reproduced from Serruya, 1978)

December or January, but in mild winters may not occur until February. The hypolimnion is depleted of oxygen from May until December, sulphide is present from September until December and ammonia also occurs in the hypolimnion during the stratification period. A general counter-clockwise circulation of water takes place in the lake.

The uppermost layer of the bottom sediments of Lake Kinneret is composed of a black, jelly-like, low density material of a thickness of 2-5 cm. The underlying sediments are grey and compact. Physical mixing of the upper layer of sediment during the winter turnover has been inferred from the complete absence of varves. Annual sedimentation rates at different stations are shown in Fig. 5.1/2. The concentrations of organic carbon in the sediments range from 1.0% in the northern tongue area to 4.8% in sediments of the central basin. Less than 10% of the carbon fixed by photosynthesis is sedimented, which suggests that P. cinctum must be extensively grazed. An unusually high C:N ratio (ca. 30:1) is found for the sediment, caused by the rapid transformation of organic nitrogen in the sediments (ammonification, nitrification) which finally lead to denitrification.

Surface and 15 cm deep sediment samples were collected (1 January 1984) from Station A (Fig. 5.1/2) by Dr. M.Gophen (Kinneret Limnological Laboratory), freeze-dried and sent to this laboratory by airmail for analysis of their free lipid compositions. The lipids of the dinoflagellate P. cinctum, the dominant member of the phytoplankton, were compared with the sedimentary lipids to indicate which dinoflagellate lipids would persist to the sediment, providing a molecular record of input

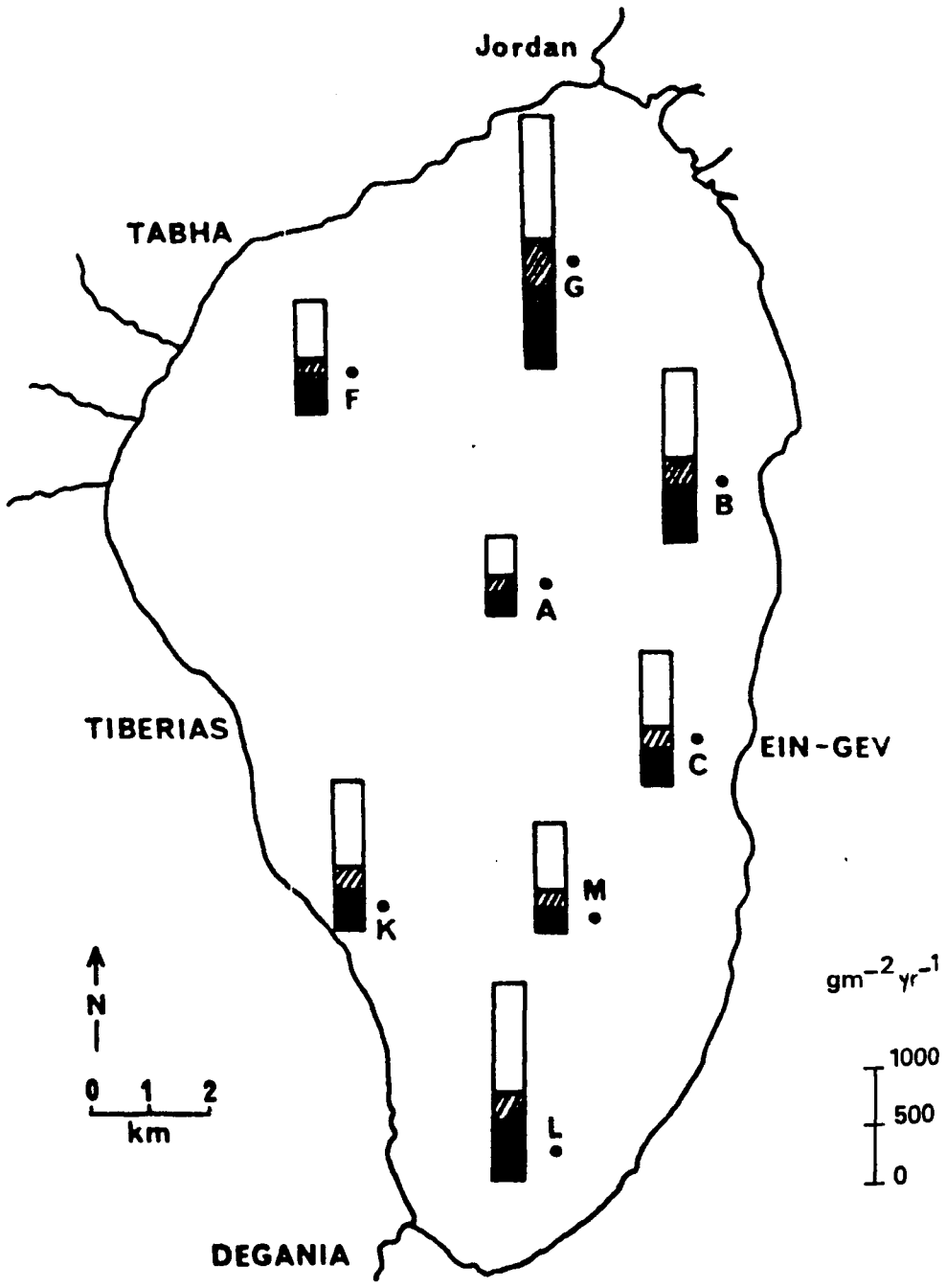


Fig. 5.1/2. Annual sedimentation rate at different stations. Results in g $m^{-2}y^{-1}$. Black area = silicates, striped area = organic matter, white area = calcium carbonates (Reproduced from Serruya, 1978)

from this organism, and which were too labile, being degraded before incorporation into the sediment. Lipids present in the sediment but not in P. cinctum must originate from some other source or by transformation of P. cinctum lipids; the distribution of such lipids should provide valuable information on the sources of, and processes affecting, the sedimentary organic matter of Lake Kinneret. Finally differences in lipid distribution between the surface and 15 cm deep sediment samples should yield information on the early stages of lipid diagenesis in Lake Kinneret sediments.

5.2 RESULTS

Freeze-dried sediment from the surface and from 15 cm deep was extracted and chromatographically separated into compound classes as shown in Fig. 5.2/1. The surface sediment gave a yellow extract producing a number of coloured bands in TLC, the least polar of which was identified as β-carotene by electronic spectroscopy. No colour was visible in the extract of the 15 cm deep sediment. CHN data is presented in Table 5.2/1. The abundance of sedimentary lipids by compound class is presented in Table 5.2/2.

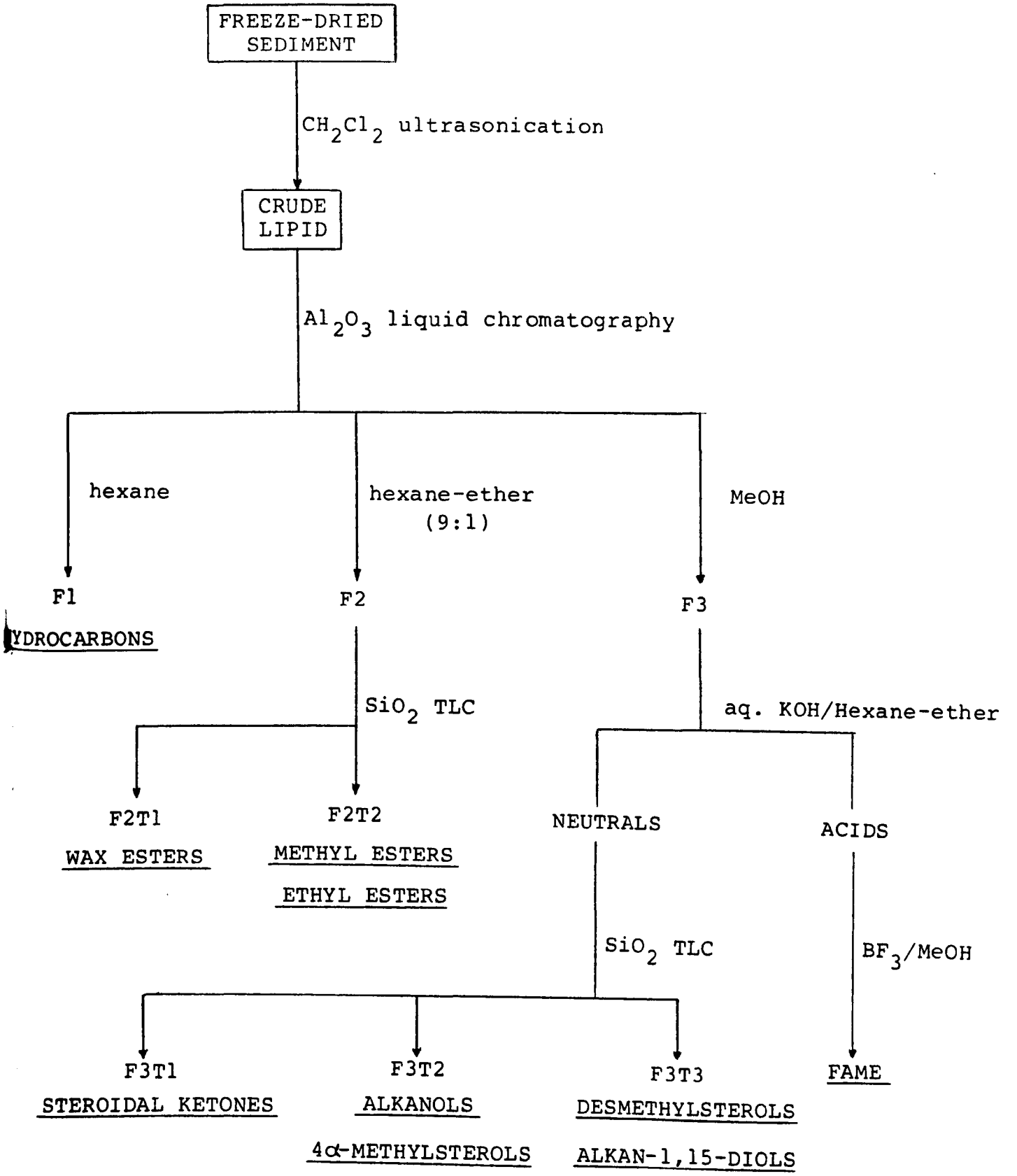


Fig. 5.2/1. Extraction and chromatographic fractionation of lipids in Lake Kinneret sediments. Compound classes underlined refer to content of various fractions as determined by GC-MS analysis.

Table 5.2/1 CHN composition[a] of Lake Kinneret surface and 15 cm deep sediment.

Sample	C	H	N	(CO_3) C
		%		
Surface	9.2, 9.4	0.71, 0.74	-	6.3, 6.3
15 cm	11.0, 11.4	0.90, 1.06	-	6.8, 6.8

(a) Values given are results of duplicate analyses.

5.2.i HYDROCARBONS

n-Alkanes (C_{17}-C_{25}, maxima C_{17} and C_{23}) were present in the surface sediment with a high CPI. The 15 cm deep sediment contained n-alkanes with a lower CPI over a wider carbon number range (C_{15}-C_{31}, maximum C_{23}) (Fig. 5.2/2). The surface sediment contained a relatively large amount of 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane (1880 ng/g freeze-dried sediment). Phytane, present in both samples, decreased in abundance between the surface and 15 cm deep samples (200 ng/g to 100 ng/g); similarly the level of hop-22(29)-ene decreased between the surface and 15 cm deep samples (720 ng/g to 510 ng/g).

5.2.ii Wax esters

Alkyl esters (C_{24}-C_{32}) containing abundant branched chain

Table 5.2/2 Abundances[(a)] of lipid compound classes isolated from Lake Kinneret sediments

Section	Hydro-carbons	Acids	Ketones	Alkanols	Desmethyl-sterols	4α-Methyl-sterols	Wax Esters
Surface	6.0	0.8	1.1	55.7	210	150	21.5
15 cm	3.5	0.4	3.0	17.9	69	87	21.0

(a) Abundance expressed in μg g dry, extracted sediment^{-1} and obtained by summing concentrations of individual components.

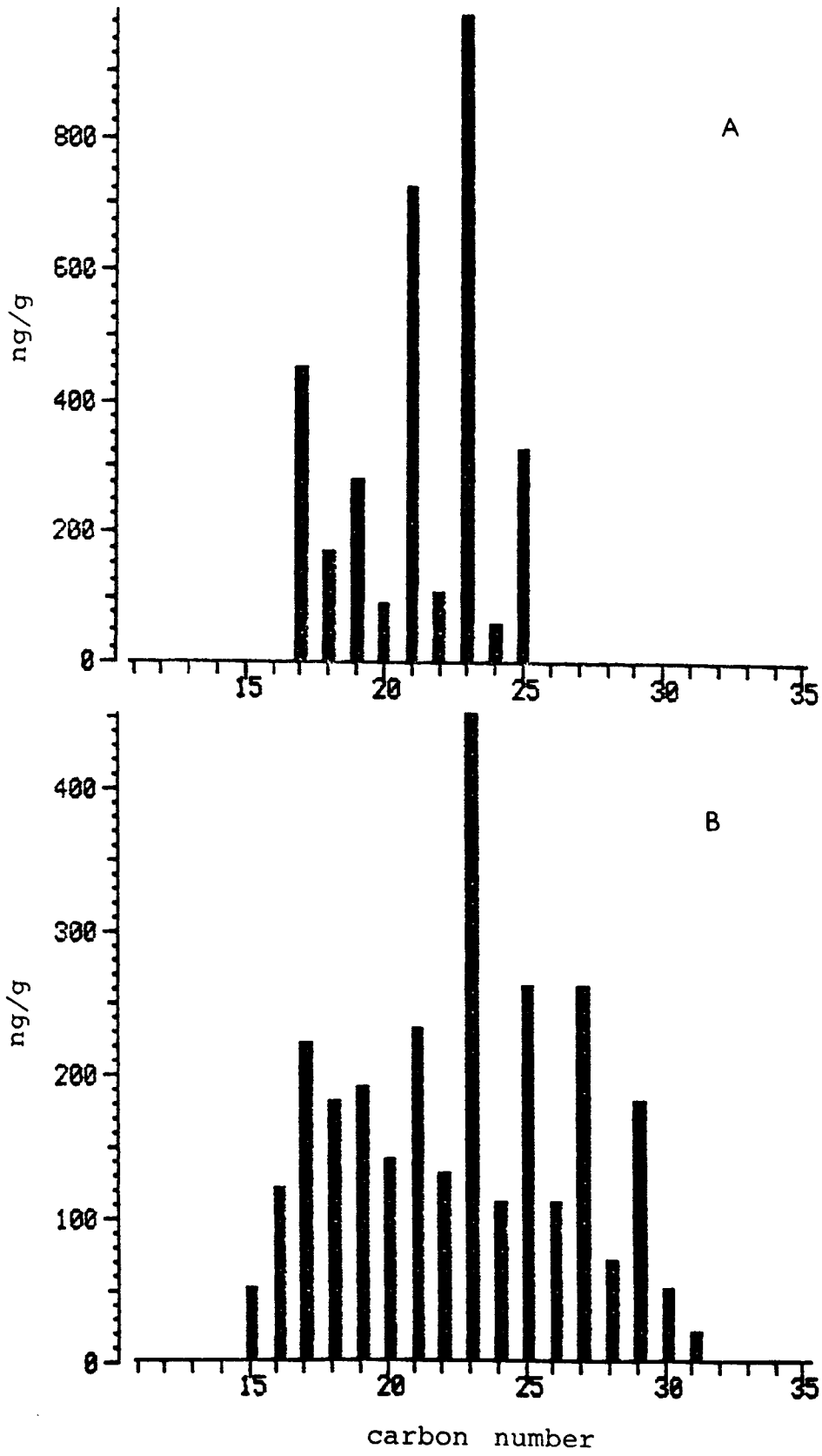


Fig. 5.2/2. Distribution of n-alkanes in Lake Kinneret sediments.
A = Surface sediments; B = 15 cm deep sediments.

Quantitation expressed as ng g dry, extracted sediment^{-1} and determined by comparison of GC peak areas with those of known amounts of C_{18} and C_{28} n-alkanes.

components, were present at relatively high concentrations (Table 5.2/3). The distribution, including molecular composition, was similar in both samples, although the alkyl esters of the 15 cm deep sediment sample contained a relatively higher proportion of branched and $<C_{27}$ components.

5.2.iii Ketones

The TLC band having the mobility of ketones (F3T1 in Fig. 5.2/1) was found to contain cyclic ketones (Table 5.2/4) and a low amount of 6,10,14-trimethylpentadecan-2-one. Retene, also present in this fraction, exhibited a striking increase in abundance between the surface and 15 cm deep samples (20 to 710 ng/g). The surface sediment contained trace levels of $\underline{n}$-C_{12}, $\underline{n}$-C_{14}, $\underline{n}$-C_{16}, $C_{18:1}$ and $\underline{n}$-C_{18} fatty acids as methyl esters, whereas the 15 cm deep sediment contained the methyl and ethyl ester of $\underline{n}$-C_{16} fatty acid (460 and 410 ng/g respectively).

5.2.iv Alcohols

a) Acyclic alcohols

Series of $\underline{n}$-alkanols were detected, maximising at C_{16} and C_{28} in the free lipids of the surface sediment and at C_{16} and C_{28} in the free lipids of the 15 cm deep sediment (Fig. 5.2/3). $\underline{Iso}$-branched C_{14}, C_{15}, C_{17} and C_{18} alkanols and $\underline{anteiso}$-branched C_{15} alkanol were present in both samples at levels approximately half that of the corresponding straight chain compound.

Table 5.2/3 Abundances of wax esters in Lake Kinneret surface and 15 cm deep sediments

Compound [a]	Alcohol-Acid [b]	% [c] Surface	% [c] 15 cm	Abundance (ng/g) [d] Surface	Abundance (ng/g) [d] 15 cm
di, (i) - 24	14 - 10		85	N.D.	100
	12 - 12		15		
(i) - 24	14 - 10	83	85	340	430
	12 - 12	17	15		
(n) - 24	14 - 10	55	52	480	500
	15 - 9	26	23		
	13 - 11	19	7		
	16 - 8	0	13		
	12 - 12	0	5		
(i) - 25	15 - 10	44	50	490	550
	13 - 12	24	18		
	14 - 11	22	17		
	16 - 9	10	15		
(n) - 25	15 - 10	47	50	820	740
	14 - 11	26	21		
	16 - 9	15	21		
	13 - 12	12	8		
di, (i) - 26	16 - 10	57	54	700	1070
	14 - 12	43	46		
(i) - 26	16 - 10	52	66	1320	2430
	14 - 12	48	34		
(n) - 26	16 - 10	49	63	2360	2440
	14 - 12	31	23		
	15 - 11	20	14		
di, (i) - 27	16 - 11		64	N.D.	90
			36		
(i, ai) - 27	16 - 11		37	N.D.	100
	14 - 13		23		
	15 - 12		21		
	17 - 10		19		
(i) - 27	15 - 12	47	43	1590	1070
	16 - 11	30	33		
	17 - 10	10	16		
	14 - 13	13	9		
(ai) - 27	16 - 11		45	N.D.	190
	17 - 10		22		
	14 - 13		19		
	15 - 12		14		
(n) - 27	15 - 12	40	38	1940	1290
	16 - 11	38	38		
	17 - 10	10	15		
	14 - 13	12	9		
di, (i) - 28	16 - 12	84	90	1700	1450
	14 - 14	16	10		
(i) - 28	16 - 12	85	93	3110	2670
	14 - 14	12	7		
	15 - 13	3	0		
(n) - 28	16 - 12	75	83	3030	2480
	15 - 13	11	8		
	17 - 11	7	5		
	14 - 14	7	4		

Continued....

Table 5.2/3 Abundances of wax ester in Lake Kinneret surface and 15 cm deep sediments - Cont'd.

Compound[a]	Alcohol-Acid[b]	% [c] Surface	% [c] 15 cm	Abundance (ng/g)[d] Surface	Abundance (ng/g)[d] 15 cm
di, (i) - 29	16 - 13		58	N.D.	70
	17 - 12		29		
	15 - 14		13		
(i, ai) - 29	16 - 13		53	N.D.	120
	17 - 12		39		
	15 - 14		6		
	14 - 15		2		
(i) - 29	17 - 12	43	46	880	560
	16 - 13	33	35		
	15 - 14	19	14		
	14 - 15	5	5		
(ai) - 29	16 - 13	51	56	290	160
	17 - 12	34	33		
	14 - 15	15	6		
	15 - 14	0	5		
(n) - 29	17 - 12	38	44	830	650
	16 - 13	38	43		
	15 - 14	12	9		
	14 - 15	5	4		
	18 - 11	7	0		
di, (i) - 30	16 - 14	59	63	300	300
	18 - 12	41	37		
(i) - 30	16 - 14	60	62	650	640
	18 - 12	40	38		
(n) - 30	16 - 14	53	54	640	590
	18 - 12	30	32		
	17 - 13	17	14		
(i) - 31	17 - 14		37	N.D.	120
	16 - 15		35		
	15 - 16		15		
	18 - 13		13		
(n) - 31	16 - 15		41	N.D.	140
	17 - 14		29		
	15 - 16		16		
	18 - 13		12		
	14 - 17				
(n) - 32	16 - 16		64	N.D.	80
	17 - 15		18		
	18 - 14		12		
	15 - 17		6		

(a) Short-hand notation is used to give information on branching pattern followed by total number of carbons, e.g., (i, ai) - 27 represents an alkyl ester containing 27 carbon atoms and possessing an iso-branched acyl or alkyl chain with the other chain anteiso-branched.

(b) Molecular composition. First number is the alkyl chain length, second is the acyl chain length.

(c) Different alcohol-acid pairings of the same total carbon number and branching pattern coeluted in GC. % molecular compositions were determined from the relative proportions of $(RCO_2)^{\cdot+}$ fragments in the mass spectrum, obtained by summing over the whole peak.

(d) Total abundance. Quantitation expressed in ng/g dry extracted sediment and determined by comparison of GC peak areas with that of a known amount of n-C_{28} alkane.

(N.D.) Not detected.

Table 5.2/4 Abundances of cyclic ketones in Lake Kinneret surface and 15 cm deep sediment

C no.[a]	Compound[b]	Structure	Abundance (ng/g)[c]	
			Surface	15 cm
27	5β(H)-Cholestan-3-one	XIV a	80	N.D.
27	5α(H)-Cholestan-3-one	XV a	90	140
28	24-Methyl-5β(H)-cholestan-3-one	XIV e	N.D.	70
28	4α-Methyl-5α(H)-cholestan-3-one	XVI a	30	110
27	Cholest-4-en-3-one	XVII a	60	N.D.
28	24-Methyl-5α(H)-cholest-22-en-3-one	XV f	N.D.	80
28	24-Methyl-5α(H)-cholestan-3-one	XV e	60	60
29	4α,24-Dimethyl-5α(H)-cholestan-3-one	XVI e	20	160
30	4α,23,24-Trimethyl-5α(H)-cholest-22-en-3-one	XVI i	280	600
29	24-Ethyl-5α(H)-cholestan-3-one	XV h	120	260
30	4α,23,24-Trimethyl-5α(H)-cholestan-3-one	XVI j	90	740
30	Olean-12-en-3-one	XXV	N.D.	110
30	Urs-12-en-3-one	XXVI	N.D.	80
30	Arborinone	XXX	110	290
27	22,29,30-Trisnorhopan-21-one	XL	20	50
29	30-Norhopan-29-one	XLI	N.D.	160
30	Hopanone	XLII	130	70

(a) Number of carbon atoms.

(b) Identifications made by comparison of mass spectra with those of standards and relative retention times.

(c) Quantitation expressed as ng g freeze-dried sediment $^{-1}$ and determined by comparison of GC peak areas with that of a standard of n-C_{28} alkane.

(N.D.) Not detected.

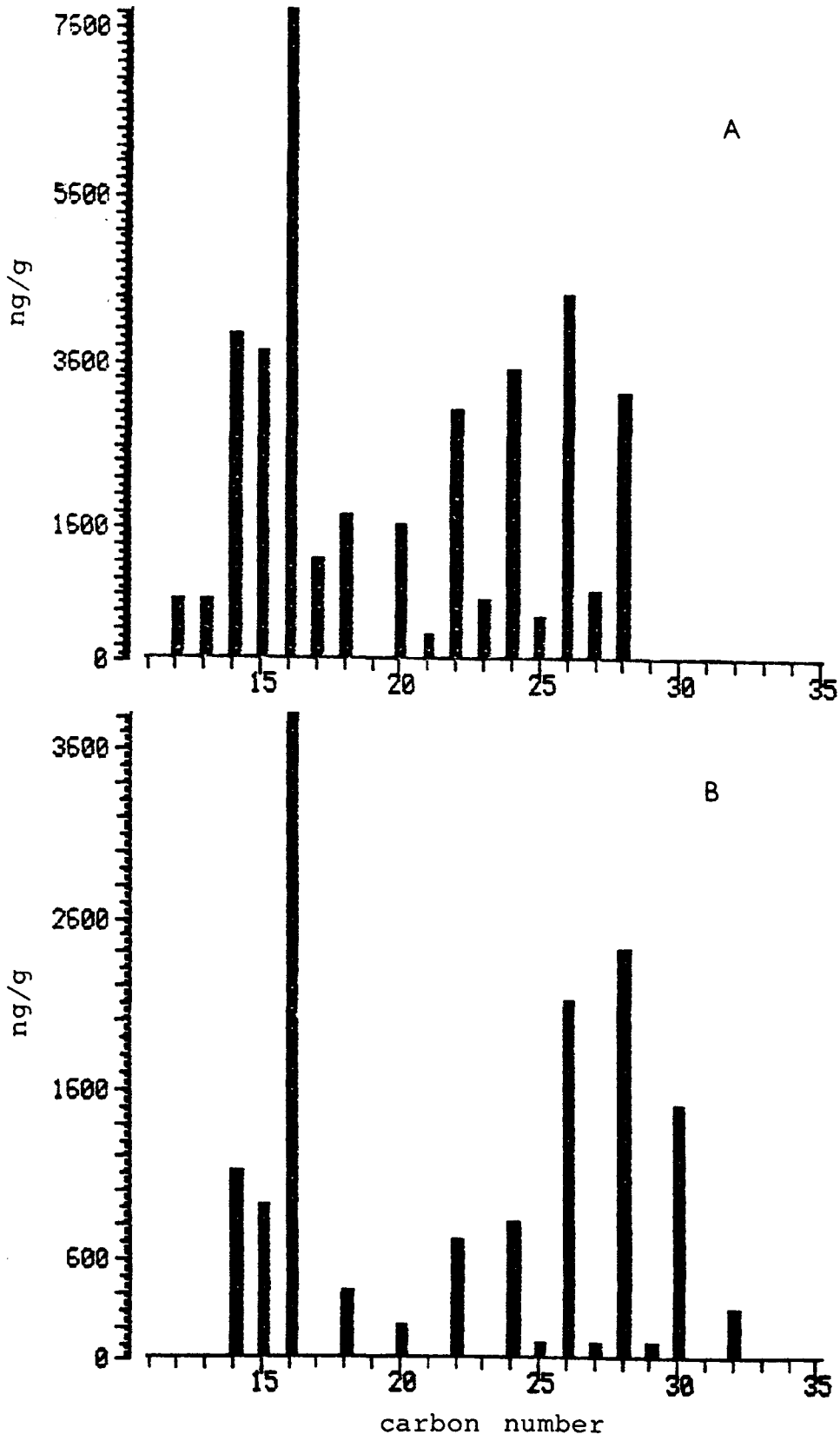


Fig. 5.2/3. Distribution of n-alkanols in Lake Kinneret sediments.
A = Surface sediment; B = 15 cm deep sediments.

Quantitation expressed as ng g dry, extracted sediment^{-1} and determined by comparison of GC peak areas with that of a known amount of n-C_{18} alkanol (TMS).

Monounsaturated alkenols were present amongst the free lipids of the surface ($C_{16:1}$ = 0.6 ug/g; $C_{18:1}$ = 2.0 and 0.8 ug/g), but were not detected in the 15 cm sediment. Phytol decreased in concentration between the surface and 15 cm deep samples (6.7 ug/g to 0.7 ug/g).

b) Cyclic alcohols

Sterols formed a major component of the free lipids of the surface and 15 cm deep sediment samples; individual abundances are presented in Table 5.2/5. A $C_{23}\Delta^5$ sterol was detected in the surficial free lipids, its mass spectrum is shown in Fig. 5.2/4. Two C_{28} stanols present in relatively high abundance eluted before 24-methylcholestanol; their mass spectra, indistinguishable from 24-methylcholestanol, demonstrated them to be 5α(H)-stan-3β-ols, a number of possibilities existing for the structure of the side-chains. Two related C_{30} desmethylsterols having one and two double bonds, respectively, were detected; their mass spectra (Fig. 5.2/5) were consistent with sterols posessing a 22,23,24-trimethyl-Δ^{22}- side-chain, the most probable side-chain from biosynthetic considerations. The second double bond was shown to be nuclear by the presence of fragment ions at m/z 213 and m/z 255 in the mass spectrum and was tentatively assigned as a Δ^7 on the basis of the longer retention time of the diunsaturated compound (as TMS) on an OV1 coated GC column. An unidentified $C_{29:1}$ 4α-methylsterol was detected amongst the free lipids of the 15 cm deep sediment; its mass spectrum and relative retention time are identical to those of such a compound detected amongst the lipids of Peridinium cinctum (Chapter 3). 4α,23,24-Trimethyl-5α(H)-cholest-17(20)-en-3β-ol was initially identified by mass spectral interpretation (Fig. 5.2/6), its mass

15 cm deep sediment

C no.[a]	Compound[b]	Structure	Abundance (µg)[d] Surface	15 cm
	Desmethylsterols			
23	C_{23} Δ^5 [d]		7.7	N.D.
26	27-Nor-5α(H)-cholestan-3β-ol		2.2	1.3
27	5β(H)-Cholestan-3β-ol	II a	1.2	tr.
27	5α(H)-Cholestan-3α-ol	IV a	0.7	tr.
27	5β(H)-Cholestan-3α-ol	V a	0.7	tr.
27	Cholest-5-en-3β-ol	VI a	12.6	2.5
27	5α(H)-Cholestan-3β-ol	III a	15.2	4.0
28	C_{28} stanol[d]		15.3	9.4
28	C_{28} stanol[d]		7.2	4.4
28	24-Methyl-5α(H)-cholest-22-en-3β-ol	III f	1.0	N.D.
28	24-Methyl-5α(H)-cholest-8(14)-en-3β-ol	X e	6.0	0.7
28	24-Methylcholest-5-en-3β-ol	VI e	16.5	1.2
28	24-Methyl-5α(H)-cholestan-3β-ol	III e	8.1	2.0
29	23,24-Dimethyl-5α(H)-cholest-22-en-3β-ol	III i	4.7	5.0
29	24-Ethylcholesta-5,22-dien-3β-ol	VI g	9.4	3.2
29	24-Ethyl-5α(H)-cholest-22-en-3β-ol	III g	6.0	1.8
28	24-Methyl-5α(H)-cholest-7-en-3β-ol	VIII e	6.4	1.7
29	24-Ethyl-5α(H)-cholesta-7,22-dien-3β-ol	VIII g	17.8	6.0
29	24-Ethylcholest-5-en-3β-ol	VI h	28.2	6.0
29	24-Ethyl-5α(H)-cholestan-3β-ol	III h	35.0	11.0
30	22,23,24-Trimethyl-5α(H)-cholest-22-en-3β-ol[d]	III o	N.D.	6.3
29	24-Ethyl-5α(H)-cholest-7-en-3β-ol	VIII h	6.9	2.0
30	22,23,24-Trimethylcholesta-7,22-dien-3β-ol[d]	VIII o	1.2	0.5
	4α-Methylsterols			
28	4α-Methyl-5α(H)-cholest-8(14)-en-3β-ol	XI a	1.1	tr.
28	4α-Methyl-5α(H)-cholestan-3β-ol	XII a	4.8	2.9
29	4α,24-Dimethyl-5α(H)-cholest-22-en-3β-ol	XII f	6.0	2.1
29	$C_{29:1}$ 4 -methylsterol[d]		N.D.	1.0
29	4α,24-Dimethyl-5α(H)-cholestan-3β-ol	XII e	tr.	4.5
30	4α,23,24-Trimethylcholesta-5,22-dien-3β-ol	XIII i	} 107.8	} 45.9
30	4α,23,24-Trimethyl-5α(H)-cholest-22-en-3β-ol	XII i		
30	4α,23,24-Trimethyl-5α(H)-cholest-17(20)-en-3β-ol[e]	XII l	3.0	2.6
30	4α,23,24-Trimethyl-5α(H)-cholestan-3β-ol	XII j	22.1	23.9
31	22,23-Methylene-4α,23,24-trimethylcholest-5-en-3β-ol	XIII m	3.5	2.6
31	22,23-Methylene-4α,23,24-trimethyl-5α(H)-cholestan-3β-ol	XII m	1.5	1.1

(a) Number of carbon atoms.

(b) Analysed as TMS ethers, identifications made by a combination of comparison of mass spectra with those of standards (e.g. Brooks et al. 1968), mass spectral interpretation and relative retention times.

(c) Quantitation expressed as µg/g freeze-dried sediment and determined by comparison of GC peak area with that of a standard of cholesterol (TMS). Where peaks overlapped or coeluted abundances were estimated from the mass spectrum.

(d) Novel sterols or tentative assignments based solely on mass spectral interpretation, see text for details.

(e) Identification confirmed by cochromatography with an authentic standard (donated by Dr. W.C.M.C. Kokke).

(N.D.) Not detected. (tr.) Trace levels present.

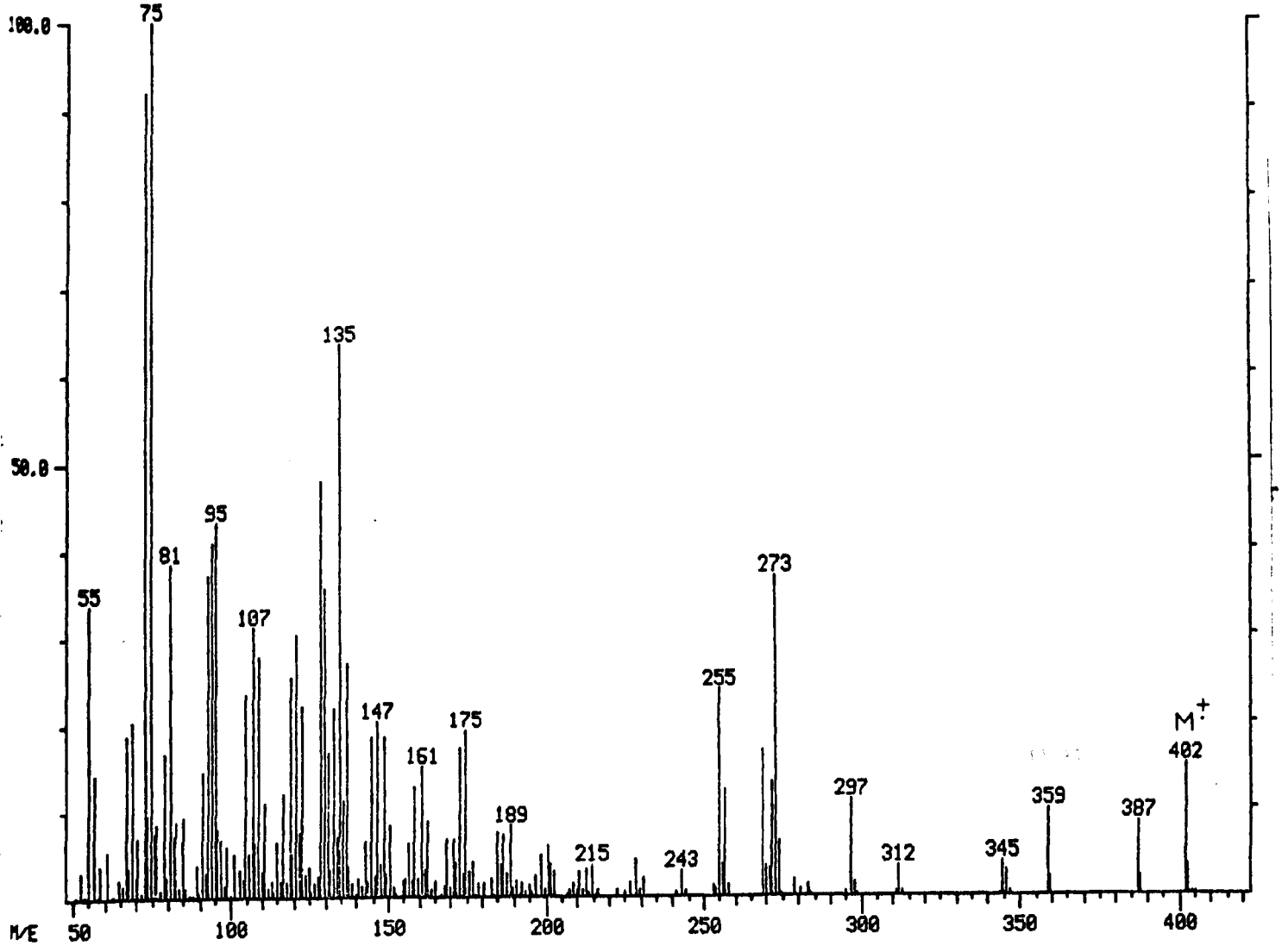
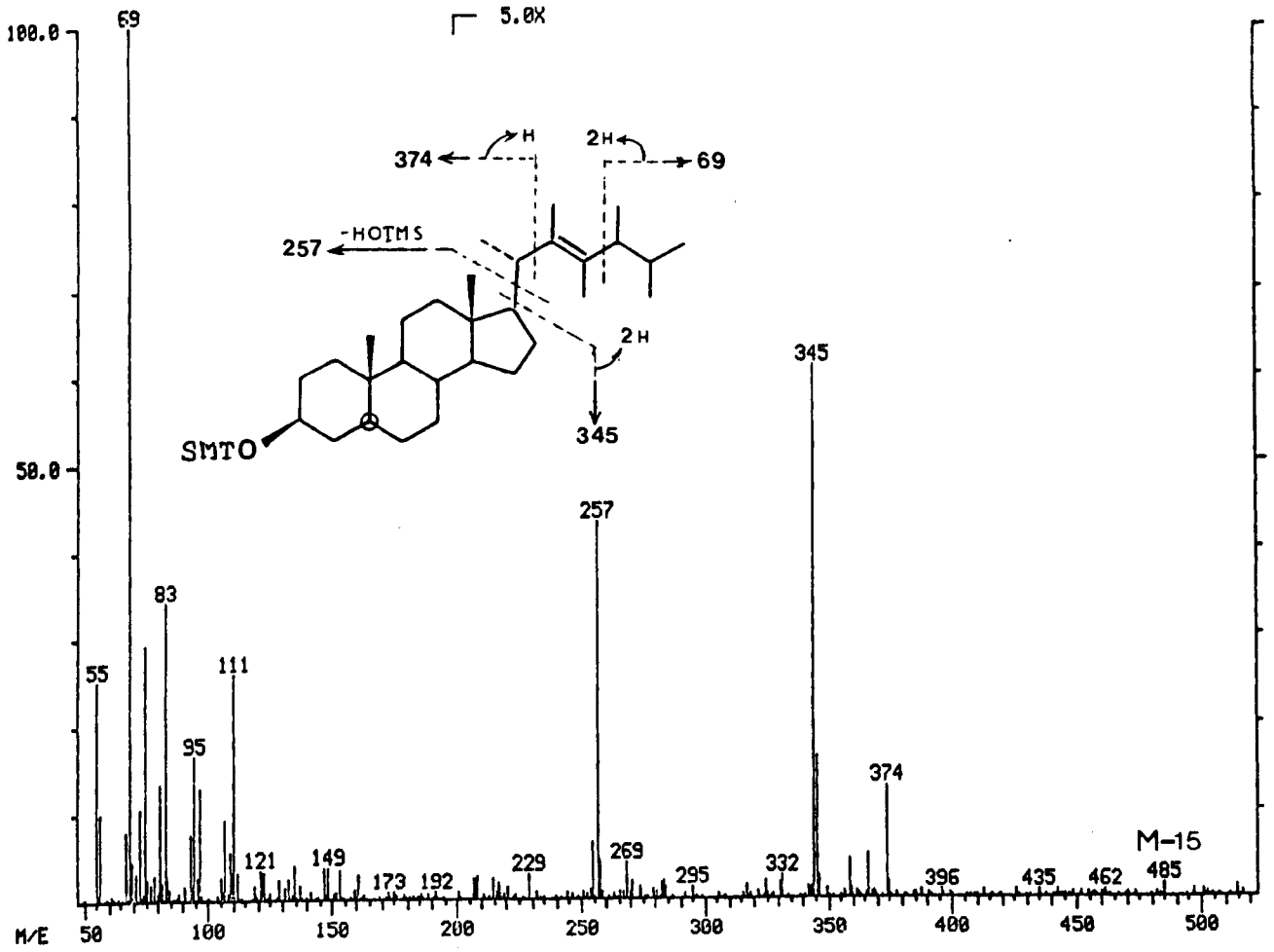


Fig. 5.2/4. Mass spectrum of C_{23} Δ^5 sterol (TMS) isolated from Lake Kinneret surface sediments.



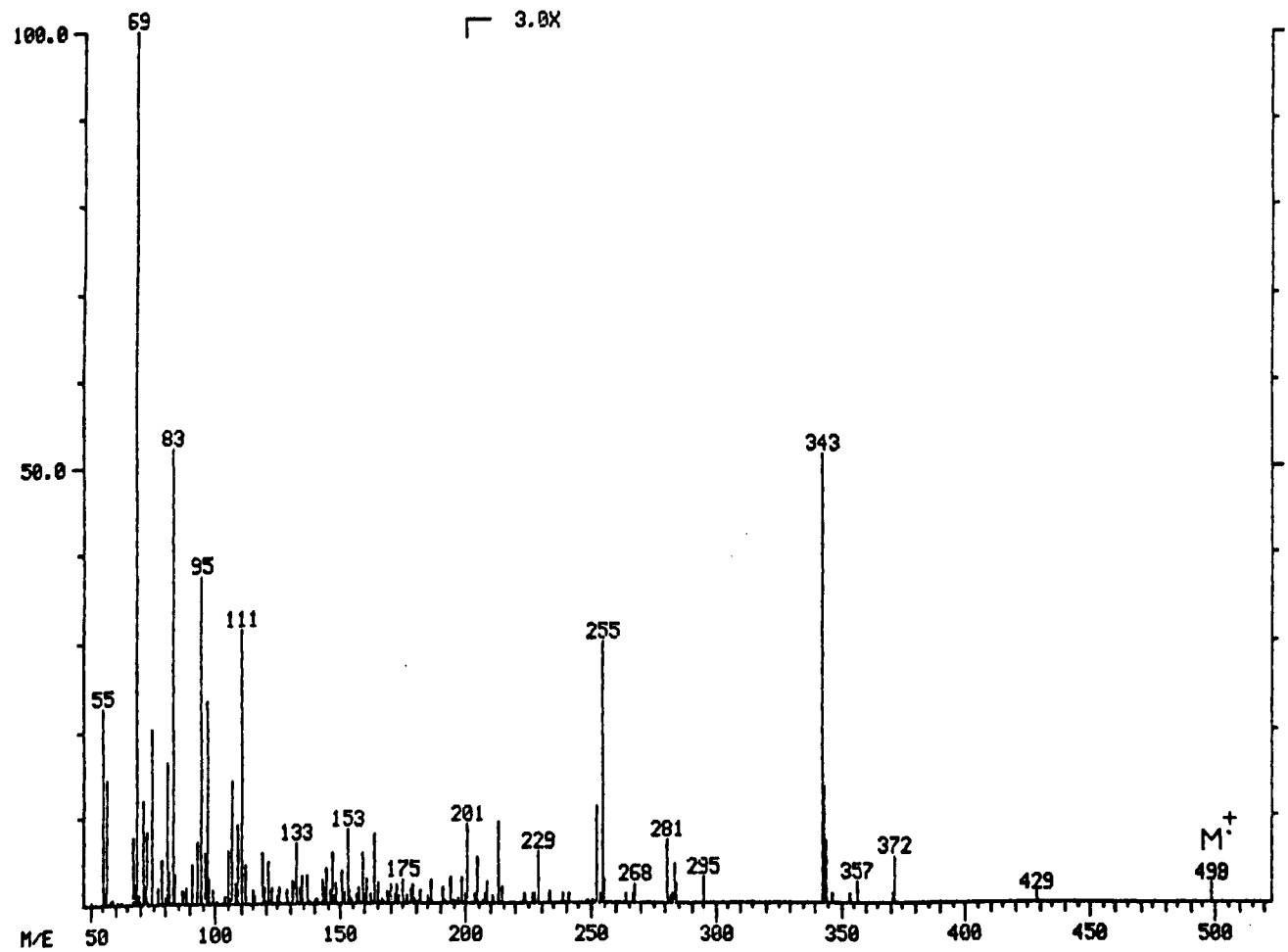
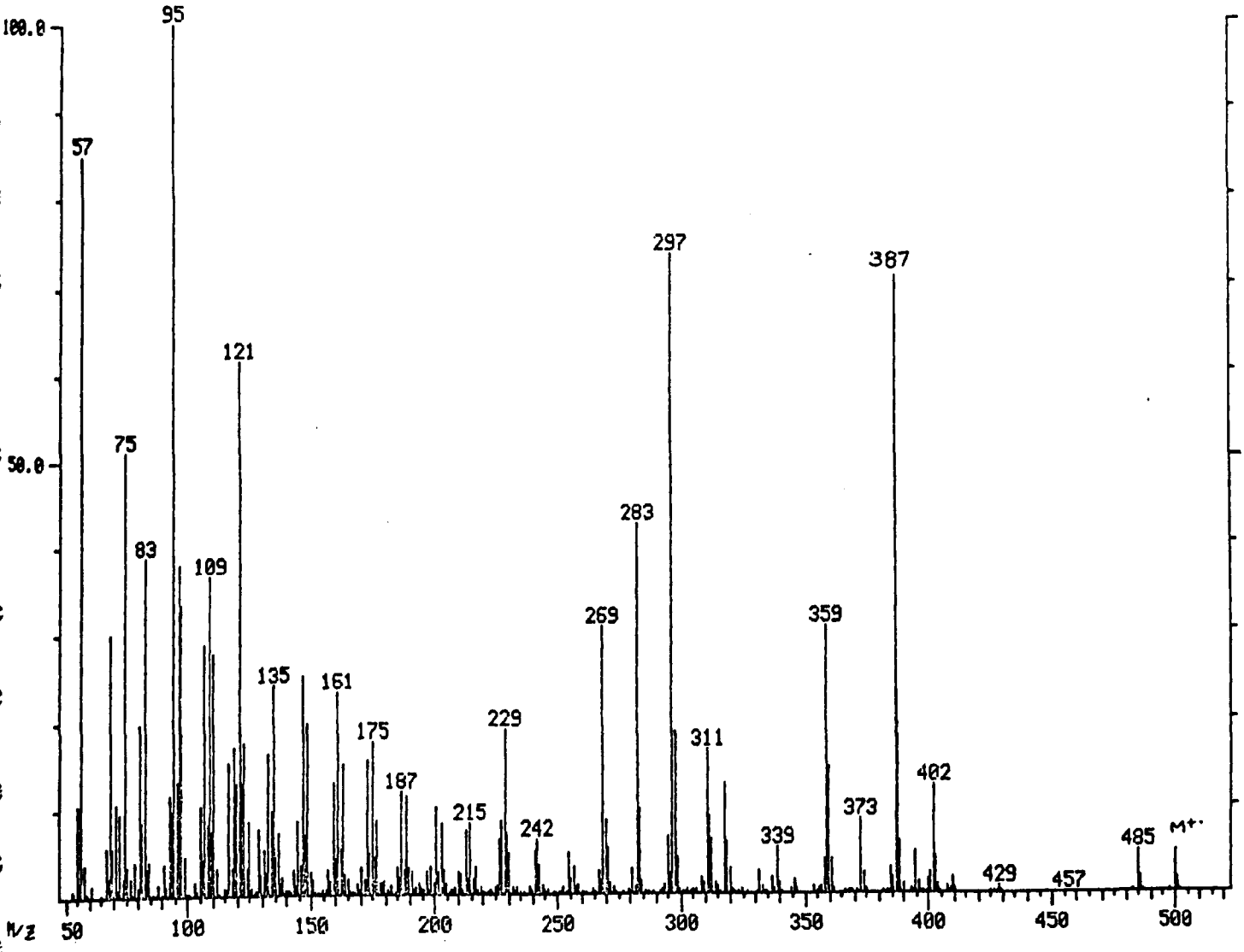


Fig. 5.2/5. Mass spectra of sterols (TMS) tentatively identified as:
(A) 22,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol and
(B) 22,23,24-trimethyl-5α(H)-cholesta-7,22-dien-3β-ol.



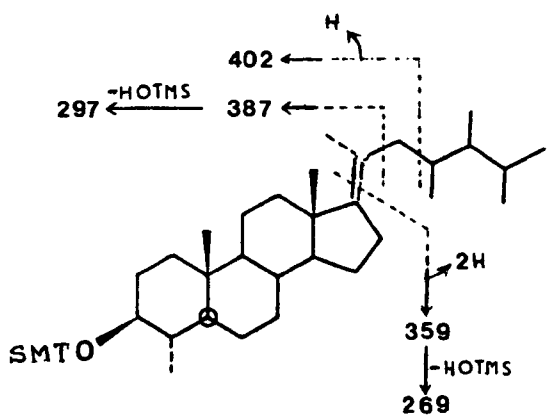


Fig. 5.2/6. Mass spectrum of peridinosterol (TMS) and proposed fragmentation

spectrum and relative retention time as TMS ether being consistent with reported data on the free sterol (Withers et al., 1979a; Swenson et al., 1980). This proposed assignment was subsequently confirmed by cochromatography with an authentic standard of the compound (donated by Dr. W.C.M.C.Kokke, Smith, Kline and French).

In addition to sterols, a number of pentacyclic alcohols were identified; identifications and abundances are presented in Table 5.2/6.

c) Alkan-1,15-diols

C_{30} and C_{32} alkan-1,15-diols were detected in the surface (1.4 and 2.2 ug/g respectively) and 15 cm deep sediments (1.6 and 0.9 ug/g respectively). Identifications were made by comparison of mass spectra with published data (De Leeuw et al., 1981).

5.2.v Carboxylic acids

Acids were analysed as methyl esters. n-C_{14-18} fatty acids (maximum C_{16}= 360 ng/g) were present amongst the free lipids of the surface sediment; additionally, trace amounts of n-$C_{20,22,24,26,28}$ fatty acids and iso- and anteiso-branched C_{15} fatty acids were detected. The 15 cm deep sediment contained n-C_{16} and n-C_{18} fatty acids (370 and 40 ng/g respectively) and trace amounts of $C_{20,22,24,26,28}$ fatty acids. $C_{16:1,18:2,18:1}$ alkenoic acids (50, 30 and 230 ng/g) were isolated from the surface sediment; the 15 cm sediment sample contained the $C_{18:1}$ alkenoic acid (30 ng/g). Hydroxy acids were not detected amongst

Table 5.2/6 Abundances of pentacyclic alcohols in Lake Kinneret surface and 15 cm deep sediment

C no.[a]	Compound[b]	Structure	Abundance[c] (µg/g)	
			Surface	15 cm
30	Olean-12-en-3β-ol	XXI	N.D.	0.4
30	Isoarborinol	XXIII	1.1	2.9
30	17α(H),21β(H)-hopan-29-ol		6.0	2.4
30	17β(H),21β(H)-hopan-29-ol	XXXVII	3.7	0.6
32	17α(H),21β(H)-bishomohopan-32-ol	XXXVIII	3.6	0.9
31	17β(H),21β(H)-homohopan-31-ol		1.7	N.D.
32	17β(H),21β(H)-bishomohopan-32-ol	XXXIX	33.7	9.7

(a) Number of carbon atoms.

(b) Analysed as TMS ethers, identifications made by comparison with literature spectra (e.g. Albrecht, 1969; Dastillung et al. 1980) and relative retention times.

(c) Quantitation expressed as µg g dry, extracted sediment^{-1} and determined by comparison of GC peak areas with that of a known amount of cholesterol (TMS).

the free lipid fractions.

5.3 DISCUSSION

5.3.i Steroids

Sterols formed the most abundant compound class in the free lipids of Lake Kinneret sediment (Table 5.2/2). The presence of sterols in a very large number of organisms and the wide variety of possible structures make this compound class particularly useful as biological marker molecules in sediments. Many of the sterols present in Lake Kinneret sediment were also detected in Peridinium cinctum, the dominant member of the phytoplankton of the lake (Fig. 5.3/1). The dominance of cholesterol found in the sterols of P. cinctum was not repeated in the sediment where dinosterol and dehydrodinosterol were the most abundant sterols. Cholestanol, present at only ca. 4% of the concentration of cholesterol in P. cinctum, was more abundant than cholesterol in the sediment; similarly, 24-methylcholestanol, absent in the organism, was isolated from the sediment in a similar amount to the corresponding Δ^5-sterol. Dinostanol was relatively more abundant in the sediment than in P. cinctum, whereas peridinosterol (4α,23,24-trimethyl-5α(H)-cholest-17(20)-en-3β-ol, XIII) was less abundant. The isolation of peridinosterol from

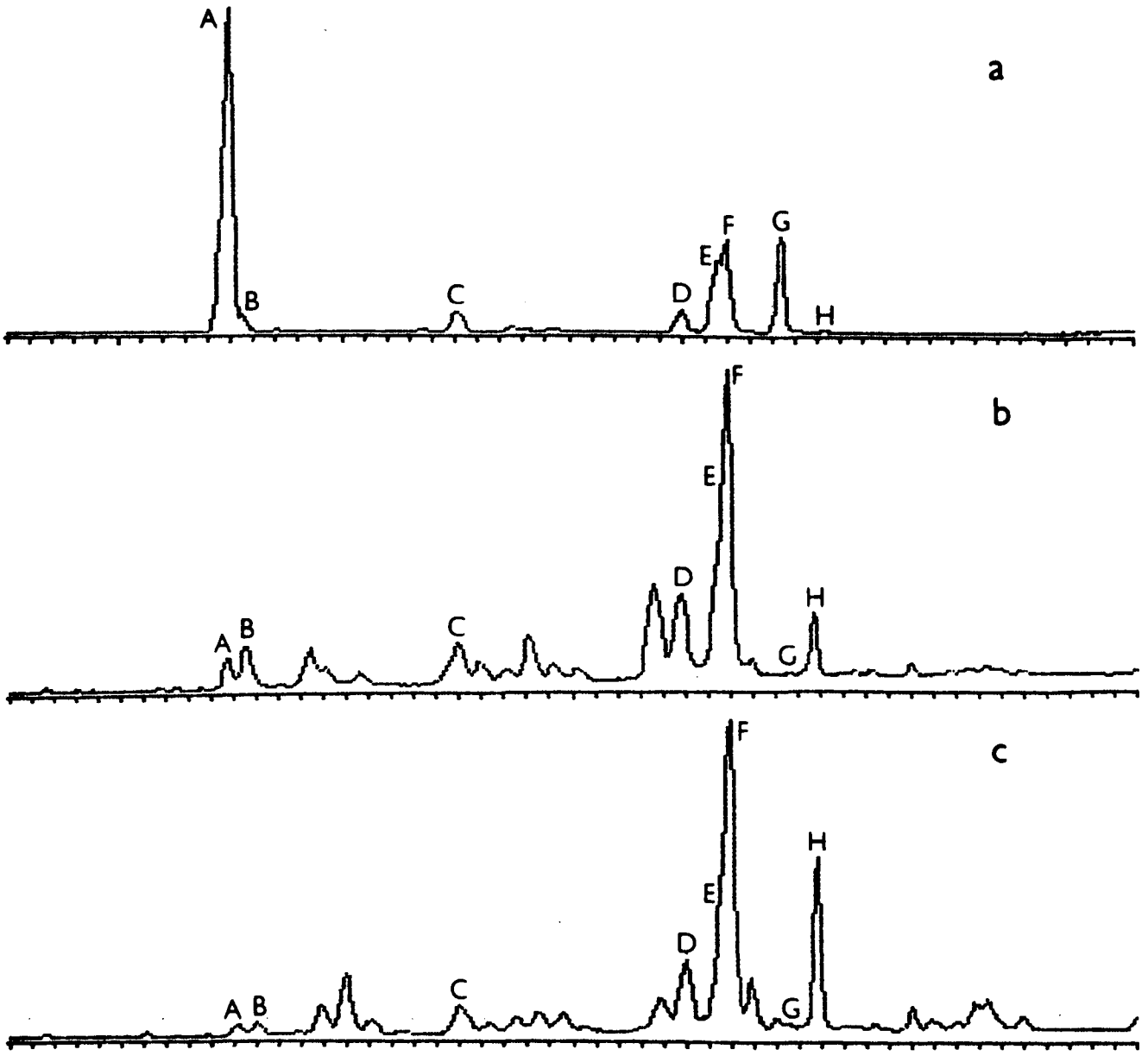


Fig. 5.3/1. Partial RIC from GC-MS analysis of the alcohols (as TMS ethers) of: a) P.cinctum; b) Lake Kinneret surface sediment; c) Lake Kinneret 15 cm sediment. Chromatograms obtained for sediment samples by combining aliquots of 4α-methylsterol and 4-desmethylsterol fractions. Identities:

A = Cholesterol
B = Cholestanol
C = 4α-methylcholestanol*
D = 4α,24-Dimethylcholestanol*
E = 4α,23,24-Trimethylcholest-5,22-dien-3β-ol (dehydrodinosterol)
F = Dinosterol
G = Peridinosterol
H = Dinostanol

* Coeluting with other sterols in the case of the sediment samples. Full identities and abundances are given in Tables 3.2/3 and 5.2/4. GC-MS conditions are given in Chapter 8.

the sediment of Lake Kinneret provides the first such recognition in a sediment.

The differences in sterol distribution between the sediments of Lake Kinneret and P. cinctum may be due to a number of factors: 1) P. cinctum may have different lipid compositions at different stages of its life-history; 2) other organisms are providing an input of lipids to the sediments; 3) lipid diagenesis will affect the distribution of lipids in the sediment; 4) transformation of lipids can take place during sedimentation through the water column (Robinson et al., 1984b; Chapter 4 this thesis). The last process may be particularly relevant as feeding studies, albeit with marine organisms, have demonstrated that zooplankton preferrentially assimilate desmethylsterols and egest 4-methylsterols, when both types of compound are present in their diet (Neal, 1984).

Chlorophyta form an important proportion of the phytoplankton during summer and autumn, they display complex sterol distributions including cholesterol and Δ^5, Δ^7 and $\Delta^{5,7}$ 24-methyl and 24-ethyl sterols (Patterson, 1967, 1974; Nes and Mckean, 1977). The presence of Δ^7-sterols in contemporary lacustrine sediments has been interpreted as reflecting an input from members of the Chlorophyta (Cranwell, 1982; Robinson et al., 1984b). Thus the C_{28} and C_{29} Δ^7-sterols and the C_{29} $\Delta^{7,22}$-sterol isolated from the sediments of Lake Kinneret probably originate from the population of Chlorophyta in the lake; the sedimentary C_{28} and C_{29} Δ^5-sterols may in part arise from the same source. Although $\Delta^{5,7}$ sterols are major sterol components of Chlorophyta, such sterols were absent from the sediments of Lake Kinneret. In Priest Pot (see Chapter 4), which also experiences a major input

of Chlorophyta derived lipids, $\Delta^{5,7}$ sterols were not preserved in the sediments, but were observed to be selectively removed within the water column before incorporation into the sediments.

Although P. cinctum dominates the dinoflagellate population of Lake Kinneret, other species do inhabit its waters (Serruya, 1978). One of these other dinoflagellates, Ceratium hirundinella, has been shown to be a potential source of some of the sterols isolated from the sediments of Lake Kinneret, e.g., 23,24-dimethyl-5α(H)-cholest-22-en-3β-ol (IIIi)(Chapter 3, this thesis).

The effects of diagenesis on the sedimentary sterols is evident from the change to a higher 5α(H):Δ^5 ratio in the deeper 15 cm depth sediment sample (1.21 to 1.60 for C_{27}; 0.51 to 1.67 for C_{28}; 1.24 to 1.83 for C_{29}). Increases in sedimentary 5α(H):Δ^5 ratios with increasing depth may be caused by either microbial hydrogenation of Δ^5-sterols (Gaskell and Eglinton, 1975) or by preferential degradation of stenols relative to stanols (Nishimura and Koyama, 1977; Nishimura, 1978). Dinostanol is much more important in the sediment than in P. cinctum (Fig. 5.3/1). The small increase in abundance of this compound between the surface and 15 cm deep sediment (Table 5.2/5) suggests that dinostanol may be formed by hydrogenation of the Δ^{22} double bond in dinosterol. The tentatively identified 22,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol (IIIo) was only present amongst the lipids of the 15 cm deep sediment, whereas the analogous $\Delta^{7,22}$-sterol was tentatively identified in both the surface and 15 cm deep sediment samples. Although the latter sterol decreased in abundance between the surface and 15 cm depth sediments, the decrease is not sufficient to explain formation of

the Δ^{22}-sterol from hydrogenation of the nuclear double bond in the $\Delta^{7,22}$-compound unless there was a relatively greater input of the $\Delta^{7,22}$ "precursor" to the 15 cm deep sediment than to the surficial sediment. The origin of these sterols is unknown, but the 22,23,24-trimethyl-Δ^{22} side-chain could be formed biosynthetically by an anti-Markovnikov opening of the cyclopropyl ring in gorgosterol/gorgostanol (cf. Rohmer et al., 1980a).

The two unidentified C_{28} 4-desmethylstanols isolated from the sediments of Lake Kinneret increased in relative abundance between the surface and 15 cm deep sediments, suggesting either an origin from within the sediment, or, more likely, a greater resistance to degradation than other sterols. Complete characterisation of the structures of these stanols requires their isolation as pure compounds in sufficient quantity for nmr studies.

4α-Methylgorgostanol (22,23-methylene-4α,23,24-trimethyl-5α(H)-cholestan-3β-ol, XIIm) has been reported to occur in sediments from the Japan Trench (Brassell et al., 1980) and has been isolated from dinoflagellate zooxanthellae (Kokke et al., 1981) and the dinoflagellates Peridinium balticum and P. foliaceum (Alam et al., 1979a; Withers et al., 1979a). In dinoflagellates 4α-methylgorgostanol may be a precursor of gorgosterol (VIm), although no dinoflagellate is known in which cyclopropanation precedes demethylation at C-4 in the biosynthesis of gorgosterol (Withers, 1983). The identification in Lake Kinneret sediments of the novel sterol 4α-methylgorgosterol (22,23-methylene-4α,23,24-trimethylcholest-5-en-3β-ol, XIIIm), provides evidence that cyclopropanation can

precede both ring saturation and demethylation at C-4 during biosynthesis. 4α-Methylgorgosterol and 4α-methylgorgostanol may originate as either byproducts of, or precursors in, the biosynthesis of gorgosterol by dinoflagellates.

Generally a decrease in abundance of sterols between the surface and 15 cm layers was observed in the sediments of Lake Kinneret (Table 5.2/2). In the absence of data on the bound sedimentary lipids, it is not known whether the decrease in free sterols is partly caused by a conversion of free to bound sterols. 4α-Methylsterols apparently survive better the early stages of lipid diagenesis than do desmethylsterols. 4α-Methylsterols have been observed to increase in relative abundance with increasing sediment depth in the marine environment (Gagosian et al., 1980), and, hence, were postulated to be more resistant to microbial degradation than desmethylsterols.

4α-Methylsteroidal ketones occuring in the surface sediments of Priest Pot have been shown to originate from direct input by dinoflagellates such as Peridinium lomnickii or Woloszynskia coronata (Robinson et al., 1984a; Chapters 3 and 4, this thesis). P. cinctum does not contain 4α-methylsteroidal ketones and this is reflected in the low sedimentary 4α-methylsteroidal ketone : 4α-methylsterol ratio for Lake Kinneret compared with Priest Pot, e.g., dinosterone/dinosterol is two orders of magnitude lower in the surface sediments of Lake Kinneret than in the 0-6 cm sediment of Priest Pot. The steroidal ketones present in Lake Kinneret may be formed by oxidation of sterols, probably microbially induced. This proposed formation is supported by the general increase in abundance of sedimentary ketones between the

surface and 15 cm deep samples in Lake Kinneret (Table 5.2/4); indeed, ketones were the only class of compound observed to increase in abundance in the lower 15 cm deep sediment. Cholest-4-en-3-one and 5α(H)-cholestan-3-one may partly originate from direct input to the sediments, as they have been isolated from C. hirundinella (Chapter 3, this thesis), a dinoflagellate reported to occur in Lake Kinneret (Serruya, 1978).

5.3.ii Other lipids

The ethyl and methyl esters detected in Lake Kinneret sediments may originate from P. cinctum, having been isolated from this organism (Chapter 3, this thesis). P. cinctum may also have contributed to the sedimentary alkanols and alkanoic acids. Heneicosahexaene, the dominant hydrocarbon of P. cinctum and other dinoflagellates (Blumer et al., 1970, 1971; Chapter 3, this thesis), was not detected in the sediment; presumably it is rapidly degraded within the aquatic environment. The distribution of sedimentary n-alkanes does not resemble the distribution observed in P. cinctum, possibly the sedimentary alkanes are derived mainly from another group of algae, such as the Chlorophyta. The presence of 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane only in the surface sediment sample, suggests that there may have been some change in input between the times of deposition of the two sediment samples, as does the lack of $>C_{28}$ alkanols in the surface sediment.

Marker compounds of terrestrial higher plants, such as C_{27}, C_{29}, C_{31} n-alkanes and certain pentacyclic triterpenoids, were of

relatively low abundance in the sediments of Lake Kinneret, consistent with the sparsity of the surrounding vegetation. The presence of 24-ethylcholesterol in the sediments may reflect algal (Patterson, 1967, 1974) as well as higher plant input; as 24R-ethylsterols generally originate from higher plants the relative proportion of allochthonous input might be determined from stereochemical analysis (e.g., Maxwell et al., 1980).

Diterpenes related in structure to abietic acid (LXIII) have been reported to occur in soils (Laflamme and Hites), lake sediments (Wakeham et al., 1980a,b; Tan and Heit, 1981; Barnes and Barnes, 1983). Conifers are a particularly rich source of abietic acid, but dehydroabietane is the only diterpene hydrocarbon repored to occur in them. In situ formation via microbial alteration of abietic acid and related diterpenes has been suggested as the source of the complete range of diterpene hydrocarbons encountered in sediments (Maxwell et al., 1971; Simoneit, 1977; Laflamme and Hites, 1978; Wakeham et al., 1980a,b). Retene (LXII), the fully aromatised hydrocarbon derived from abietic acid, is usually the dominant diterpene in lake sediments (Wakeham et al., 1980b; Tan and Heit, 1981; Barnes and Barnes, 1983). In Lake Washington, retene exhibits an increase in abundance from the sediment surface to a maximum at 6-9 cm depth, followed by a decrease with greater depth (Wakeham et al., 1980b); a similar pattern was observed in West Basin sediments of Powell Lake (Barnes and Barnes, 1983). High sedimentary retene concentrations have been proposed to be associated with reducing environments, which aid preservation and probably contribute to its in situ formation (Barnes and Barnes, 1983). In the mainly anoxic sediments of Lake Kinneret retene increased in

concentration from 20 ng/g to 710 ng/g between the surface and 15 cm sediment samples. It probably originates from *in situ* formation by microbial alteration of abietic acid, derived from conifers. Such formation is presumably aided by the presence of an anoxic hypolimnion from May until December.

Alkyl esters, in the C_{24}-C_{33} range with a low predominance of even carbon number homologues and abundant branched chain components, have been isolated from recent sediments of two productive lakes (Cranwell, 1983). The branched chain components were mainly *iso*- and *anteiso*-branched in either the alkyl or acyl chain, with minor products branched in both chains. Similar branching patterns occur widely in bacterial lipids (Parker, 1968; Kaneda, 1977), providing circumstantial evidence for a bacterial origin of the C_{24}-C_{33} sedimentary alkyl esters. Confirmatory evidence of such an origin is provided by the isolation of Quirk (1978) of ^{14}C-labelled wax esters of similar distribution and molecular composition from the incubation of ^{14}C-acetate in a fen peat. The C_{24}-C_{32} alkyl esters isolated from the sediments of Lake Kinneret have a very similar distribution and molecular composition (Table 5.2/3) to those reported by Cranwell (1983), although in the esters isolated from Lake Kinneret C_{14} alkyl is more abundant and generally there is a shorter average alkyl chain length and a corresponding longer acyl chain length. The wax esters present in the sediments of Lake Kinneret probably originate from bacteria. Small differences in distribution observed between the surface and 15 cm deep sediment samples may be due to inputs from various bacterial populations inhabiting distinct sections of the sediment. Higher plants possess even carbon number straight chain

saturated esters in the C_{32}-C_{64} region (Tulloch, 1976), the absence of such compounds from the sediments of Lake Kinneret is consistent with the generally low allochthonous input to the lake.

The hopanoids detected in Lake Kinneret sediments reflect a bacterial input (see Ourisson et al., 1979). Similarly isoarborinol and arborinone have been attributed to bacterial activity, although they have not been isolated from an organism (Brassell et al., 1983).

C_{30} and C_{32} alkan-1,15-diols have been previously reported to occur in marine sediments (De Leeuw et al., 1981; Brassell et al., 1981; Thomson et al., 1982; Smith et al., 1983). The isolation of these compounds from Lake Kinneret sediments, as well as from sediments of Priest Pot (Chapter 4) and Messel oil shale (Chapter 6), demonstrates they may also occur in lacustrine sediments. Although long chain alkan-1,15-diols have not been detected in an organism, the presence of these compounds in sediments having a significant dinoflagellate input, makes dinoflagellates a likely source. The absence of such compounds in the free lipids of P. cinctum implies that, if dinoflagellates are the source, alkan-1,15-diols may be present in a bound form and only released during lipid diagenesis in the sediment. The C_{30} and C_{32} alkan-15-one-1-ols detected in marine sediments (De Leeuw et al., 1981; Smith et al., 1983) presumably arise by microbial oxidation of the corresponding diols during diagenesis.

5.4 CONCLUSIONS

Surficial and 15 cm deep sediment samples from Lake Kinneret, Israel, have been analysed for their free lipid content. Comparison of the sedimentary lipid distributions with those of P. cinctum, the dominant member of the lake's phytoplankton, allowed a number of conclusions to be drawn regarding the origin and fate of lipids in the sediments of Lake Kinneret.

1) The record of input to the sediment from P. cinctum is preserved in the sedimentary lipids, and is particularly apparent in the distribution of 4α-methylsterols. Methyl and ethyl esters of fatty acids, present in P. cinctum, were also detected in the sediments, but heneicosahexaene, the dominant hydrocarbon of this dinoflagellate, was absent from the bottom sediments of Lake Kinneret, presumably being rapidly degraded within the water column and the top layer of sediment.

2) The abundance of 24-ethyl and 24-methyl Δ^5 and Δ^7 sterols in the sediment of Lake Kinneret suggests that there is a significant input from the population of Chlorophyta inhabiting the lake.

3) Bacteria are apparently a major source of sedimentary lipids in Lake Kinneret, based on the sedimentary content of hopanoids and alkyl esters.

4) Higher plants are not a major source of lipids in Lake Kinneret sediment, reflecting the sparsity of the surrounding vegetation.

5) Although there may have been small differences in input, the two sediment sections studied appear to have been deposited under similar biological and chemical conditions.

6) Transformation of lipids commences within the water column and continues in the bottom sediments of Lake Kinneret, modified by the largely anoxic conditions. Thus, $5\alpha(H):\Delta^5$ sterol ratios are greater in the deeper sediment section, presumably due to microbial hydrogenation and/or to preferential degradation of stenols; $\Delta^{5,7}$ sterols, constituents of Chlorophyta, are not preserved in the sediment; all compound classes, with the exception of ketones and wax esters, exhibit a marked decrease in abundance of free lipids in the lower sediment section. 4α-Methylsterols are relatively more abundant in the surface sediment sample than in P. cinctum, and are further enhanced in the 15 cm deep sediment sample, implying that 4α-methylsterols are more resistant to degradation than are desmethylsterols. Unsaturated alcohols are preserved in the surface sediments, presumably aided by the anoxic conditions; however, alkenols are absent from the 15 cm deep sediment.

7) Retene increases in abundance between the surface and 15 cm deep sediments, probably due to in situ formation, under anoxic conditions, from abietic acid or its derivatives.

8) Steroidal ketones and 4α-methylsteroidal ketones were isolated from the sediments. The absence of all but two of these ketones from P. cinctum, and the increase in abundance between the surface and 15 cm deep sediment samples, suggest that sedimentary steroidal ketones in Lake Kinneret are formed by microbial oxidation of the corresponding sterols.

9) A number of novel compounds or lipids not previously reported to occur in contemporary lake sediments have been detected. C_{30} and C_{32} alkan-1,15-diols, previously only reported to occur in marine sediments, have been isolated from Lake

Kinneret surface and 15 cm deep sediments. The novel sterol 4α-methylgorgosterol has been isolated from a natural sample for the first time and provides new information regarding the biosynthesis of 22,23-methylene containing sterols by dinoflagellates. Two novel C_{30} sterols were isolated from Lake Kinneret sediments and tentatively identified as 22,23,24-trimethylcholesta- 7,22-dien-3B-ol and its Δ^7 analogue; their origin may be related to gorgosterol. Two C_{28} stanols of unknown side-chain structure were present in the sediments of Lake Kinneret as major components of the sterols, increasing in relative abundance between the surface and 15 cm deep sediment samples. The origin of these stanols is also unknown.

CHAPTER SIX

MESSEL OIL SHALE: A REAPPRAISAL

6.1 INTRODUCTION

The Messel oil shale is an organic-rich Eocene sediment (ca. $50x10^6$ years old) situated 9 Km north-east of Darmstadt, Germany (Fig. 6.1/1). The shale is preserved in a basin 1000 m long and 700 m wide and the organic-rich layer has not been buried deeper than about 200 m (Fig. 6.1/2) (Matthes, 1968). Deposition probably took place in a series of shallow swampy lakes linked by slow-moving fluvial systems; pollen data (Sittler, 1968) for the Messel shale shows that Pteridophyta, Myricaceae, Fagaceae (Castaneoideae) and Cupressaceae predominated in the palaeoenvironment and, together with fossil plant information (Matthes, 1956, 1968), indicates that a hot, damp, tropical climate existed at the time of deposition. The sediment comprises mainly gyttja (sapropelic black mud) with fine sandy clay intercalations and is composed of 25% organic material, 35% inorganic material and 40% water (Matthes, 1968); the inorganic portion is mainly montmorillonite, and most of the organic carbon is present as kerogen. Mild burial conditions (probably no more than 40 °C, Kimble et al., 1974a) have resulted in exceptional preservation of organic compounds and lipids of biological origin have survived intact. Literature concerning Messel oil shale was last reviewed by Koenigswald (1980), in which nearly 50 papers, describing mainly faunal and floral remains found in Messel sediment, are described.

Previous studies of the extractable lipids of Messel have identified isoarborinol (XXIII) (Albrecht and Ourisson, 1969), arborinone (XXX), friedelin (XXIX), a series of 4α-methylstanols

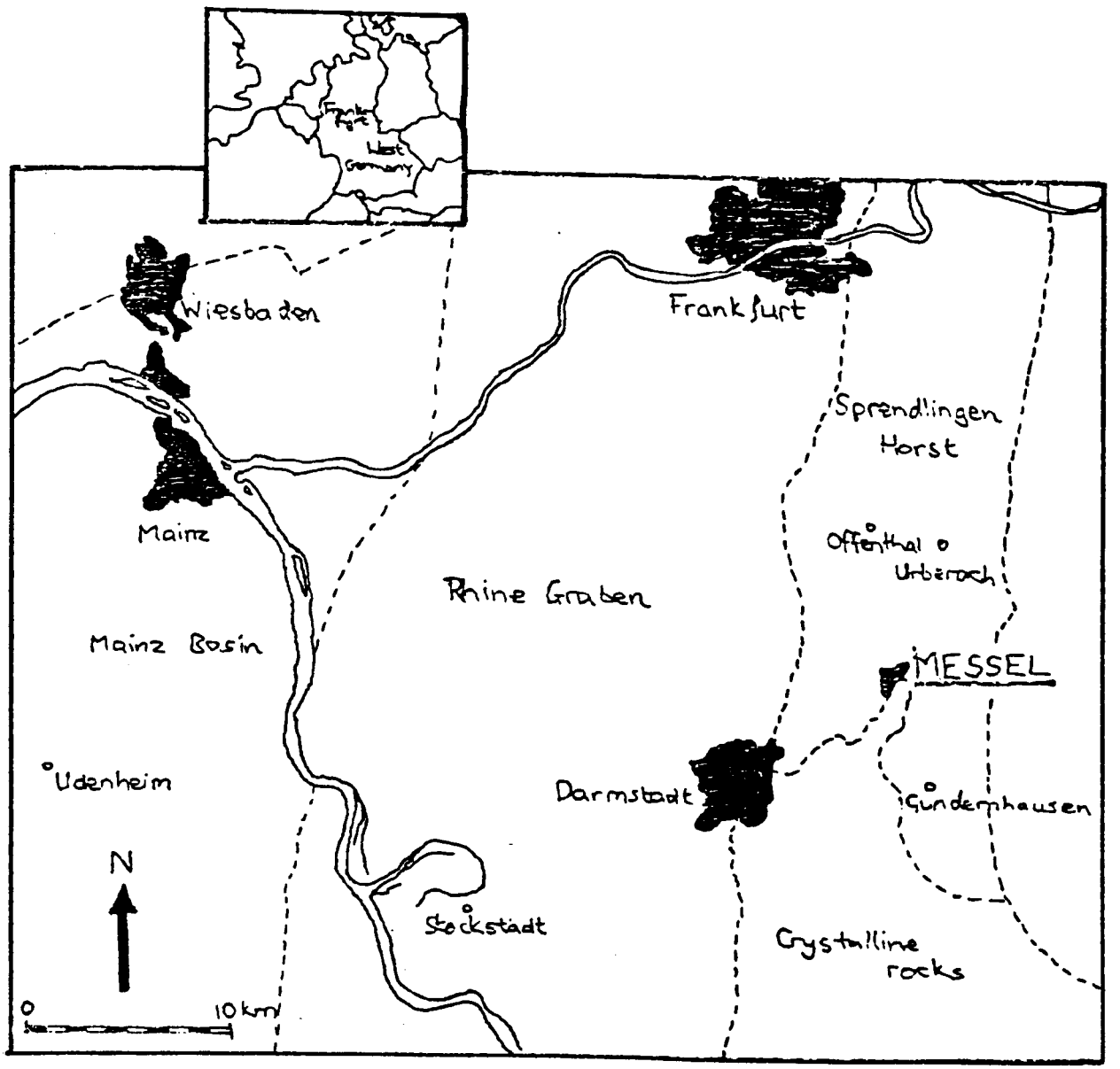


Fig. 6.1/1. The location of Messel oil shale.
(After Matthes, 1968)

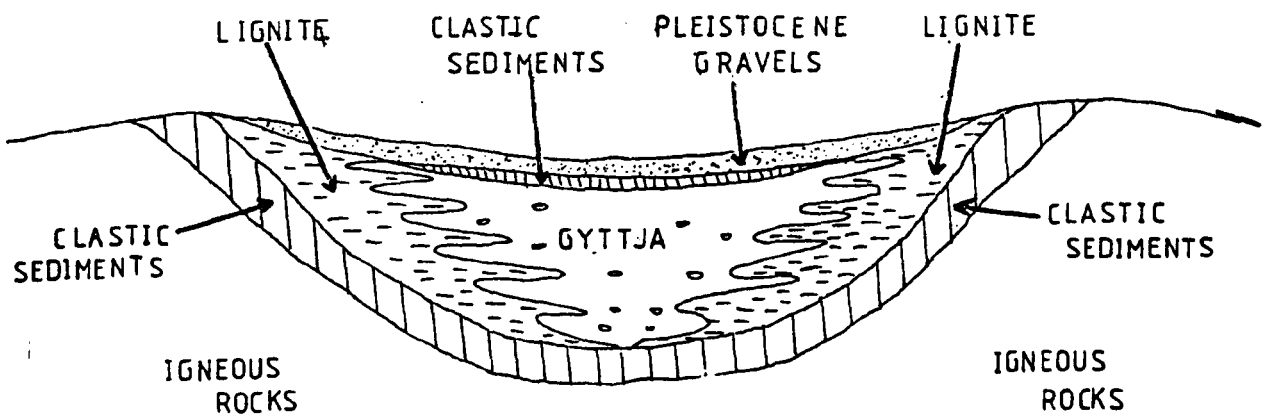


Fig. 6.1/2. Diagrammatic cross section of the Messel basin. (Reproduced from Matthes, 1968)

Lignite = brown coal; gyttja = unconsolidated, organic rich sediment.

and 4α-methylstanones, and very small quantities of phytosterols (Mattern et al., 1970). Hydrocarbons have been found to contain n-alkanes mainly in the range C_{23} to C_{33} with a CPI >6, a relatively high abundance of hopanoid hydrocarbons and a series of 4-methylsteranes in higher abundance than their 4-desmethyl analogues (Albrecht, 1969; Ensminger et al., 1972; Kimble et al., 1974a; Wardroper et al., 1977). A number of aromatic hydrocarbons have been isolated from Messel oil shale and were shown to have structures LIV to LX by synthesis of standards (Greiner et al., 1976, 1977; Spyckerelle et al., 1977a,b). Long chain α,w- and α,(w-1)-diols have been reported to be present (Habermehl and Springer, 1983b). Treatment of kerogen of Messel shale with boron tribromide, followed by reduction with lithium aluminium hydride (or deuteride), has revealed the presence of biological marker compounds for Archaebacteria (Chappe et al., 1980).

The above work carried out on the free lipids of Messel oil shale, was mainly performed before recent advances in instrumentation, i.e. before the use of high resolution capillary columns, computerised data acquisition and rapid scanning for GC-MS. Furthermore, at the time the work of Mattern et al. (1970) on the 4α-methylstanols and 4α-methylstanones was performed, such compounds were not reported to occur as significant components in organisms. Subsequently 4α-methylsteroids have been detected in a methanotrophic bacterium (Bird et al., 1971; Bouvier et al., 1976) and in a greater range in dinoflagellates (Shimizu et al., 1976; Withers et al., 1978, 1979a, b, c; Kokke et al., 1981a, b, 1982; Robinson et al., 1984a). As well as related series of three

4α-methylstanols and three 4α-methylstanones, Mattern et al. (1970) reported an unidentified 4α-methylsterol and an unidentified 4α-methylsterone, the mass spectra and GC retention times of which (Albrecht, 1969) suggested that they were dinosterol (XIIi) and dinosterone (XVIi), respectively. These compounds are now regarded as biological markers for dinoflagellate input to sediments (Boon et al., 1979; Gagosian and Smith, 1979; Brassell and Eglinton, 1983; De Leeuw et al., 1983; Robinson et al., 1984a); thus, in the light of new information on the origin of sedimentary lipids, it was thought that the Messel oil shale could profitably be reappraised using advanced analytical techniques, and that it would be a very useful extension of the work on freshwater organisms and contemporary lacustrine environments described elsewhere in this thesis. Some of the work described in this chapter (that concerning the sterols and steroidal ketones) was performed in collaboration with Mr. M.A.Allen as part of a final year undergraduate project.

Recent work (Habermehl and Hundrieser, 1983a) performed independently of our own, and published after the commencement of this study, reported the occurrence in Messel oil shale of a number of 4α-methylsterols and 4α-methylsteroidal ketones (summarised in Table 6.1/1), including dinosterol and 4α-methylgorgostanol (XIIn). The occurrence of these compounds was proposed to be due to dinoflagellate activity at the time of deposition.

Table 6.1/1 Steroidal compounds reported to be present in Messel oil shale by Habermehl and Hundrieser (1983)

Compound	Structure
22,23,24,25,26,27-Hexanor —4α-methylcholestan-3β-ol	
25,26,27-Trisnor —4α-methylcholestan-3β-ol	
4α-Methylcholestan-3β-ol	XII a
4α,24-Dimethylcholestan-3β-ol	XII e
4α,23,24-Trimethylcholest-22-en-3β-ol	XII i
4α,Methyl,24-ethylcholestan-3β-ol	XII h
22,23-Methylene-4α,23,24-trimethyl-5α-cholestan-3β-ol	XII m
22,23,24,25,26,27-Hexanor —4α-methylcholestan-3-one	
25,26,27-Trisnor —4α-methylcholestan-3-one	
4α-Methylcholestan-3-one	XVI a
4α,24-Dimethylcholestan-3-one	XVI e
4α,23,24-Trimethylcholest-22-en-3-one	XVI i
4α-Methyl,24-ethylcholestan-3-one	XVI h

6.2 RESULTS

Details of the history of the sample of Messel shale used in this study are uncertain. The shale (ca. 750 g) had been stored in the dark in two closed glass jars marked "Messel non lavé" . Extraction (Fig.6.2/1) yielded soluble organic material totalling 3% by weight of the shale. Separation into compound classes was achieved by alumina column chromatography followed by silica TLC (Fig. 6.2/1). CHN analysis of the shale is presented in Table 6.2/1.

Table 6.2/1. CHN* composition of Messel oil shale.

ORGANIC %			INORGANIC %
C %	H %	N %	Carbonate
30.0	4.0	0.4	1.0

(*) Presented as % by weight of dry sediment.

Absolute quantitation was not determined on the lipid extract, however, relative abundances were estimated from FID response in GC as:

Alkan-diols >4α-methylsterols >alkanols >cyclic ketones >alkan-2-ones >methyl esters >aliphatic hydrocarbons >mid-chain ketones >aromatic hydrocarbons.

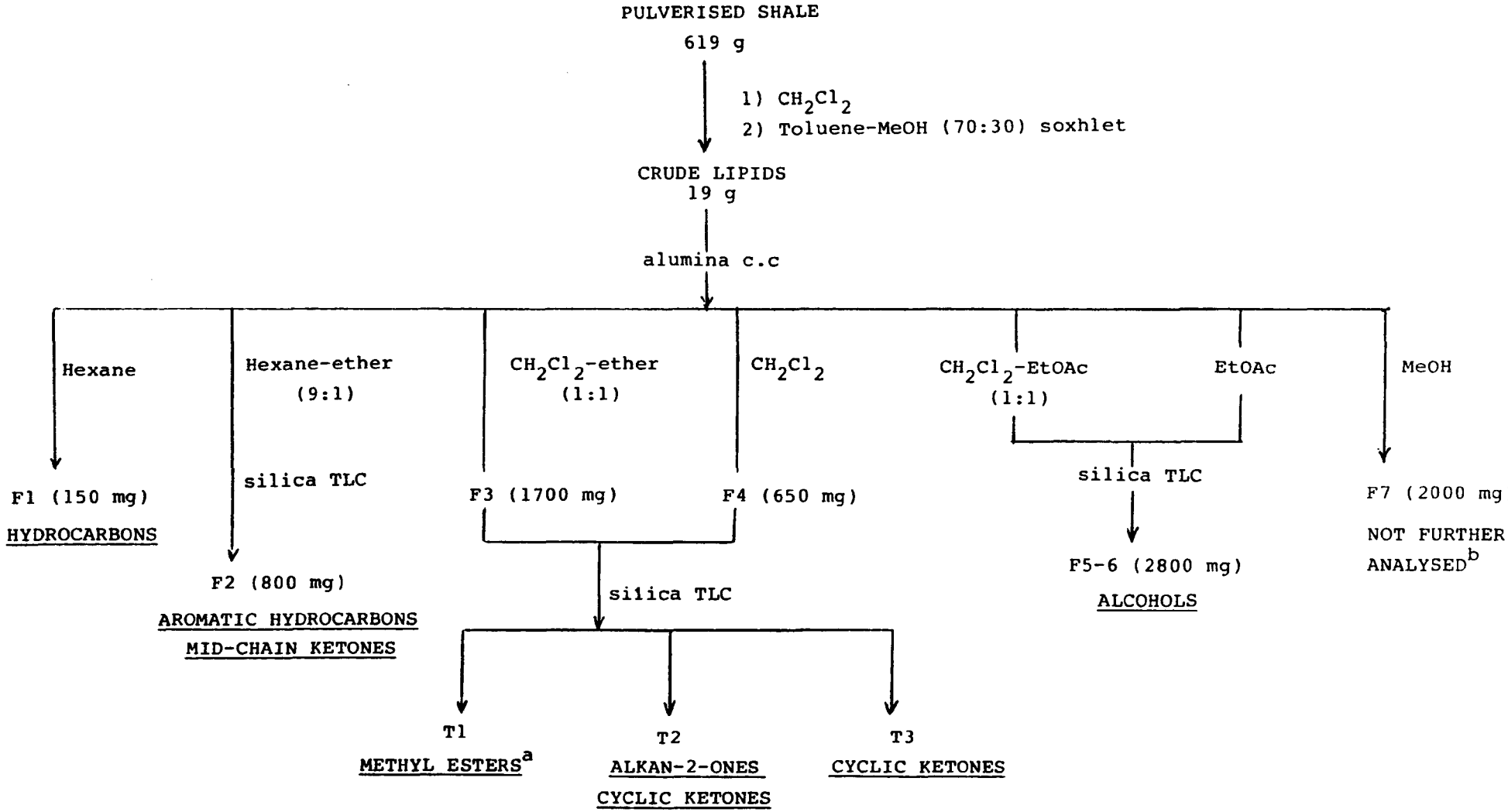


Fig. 6.2/1. Schematic diagram showing extraction and chromatographic separation of lipids in Messel oil shale. Compound classes underlined refers to lipid content of various fractions as subsequently determined by GC-MS analysis. Some material was not eluted from the alumina column by the solvents used.

(a) Also contained an aromatic hopanoid (see text).

(b) Preliminary analysis revealed that the majority of the material could not be gas chromatographed.

6.2.i Hydrocarbons

a) Acyclic hydrocarbons

n-Alkanes with a CPI of 4.6 were detected over the range C_{14} to C_{33}, maximising at C_{23} and C_{27} (Fig. 6.2/2). Isoprenoid alkanes were present in the relative order of abundance $C_{16} > C_{15} > C_{20} > C_{19} > C_{18}$; individual concentrations were < 8% of the concentration of the n-C_{23} alkane.

b) Cyclic hydrocarbons

A series of hopanoid hydrocarbons were detected, their distribution is shown by means of a m/z 191 mass fragmentogram in Fig. 6.2/3. No steroidal or 4α-methylsteroidal hydrocarbons were detected in this sample of Messel shale.

c) Aromatic hydrocarbons

Three aromatic hydrocarbons were identified in a fraction (F2 in Fig. 6.2/1) also containing mid-chain ketones. The most abundant earliest eluting compound was tentatively identified as (3,3′,4,4′-tetramethyl)-1,1′-biphenyl by comparison of its mass spectrum with that of a library entry, the second eluting, least abundant compound was identified as having structure LX by comparison of its mass spectrum with that of a standard (Spyckerelle et al., 1977a, b) and the third compound was similarly identified as having structure LVI. One additional aromatic hydrocarbon was detected in the same fraction as the methyl esters (T1 in Fig. 6.2/1). Its mass spectrum indicated it to have a molecular formula $C_{24}H_{22}$ and was identical with that of

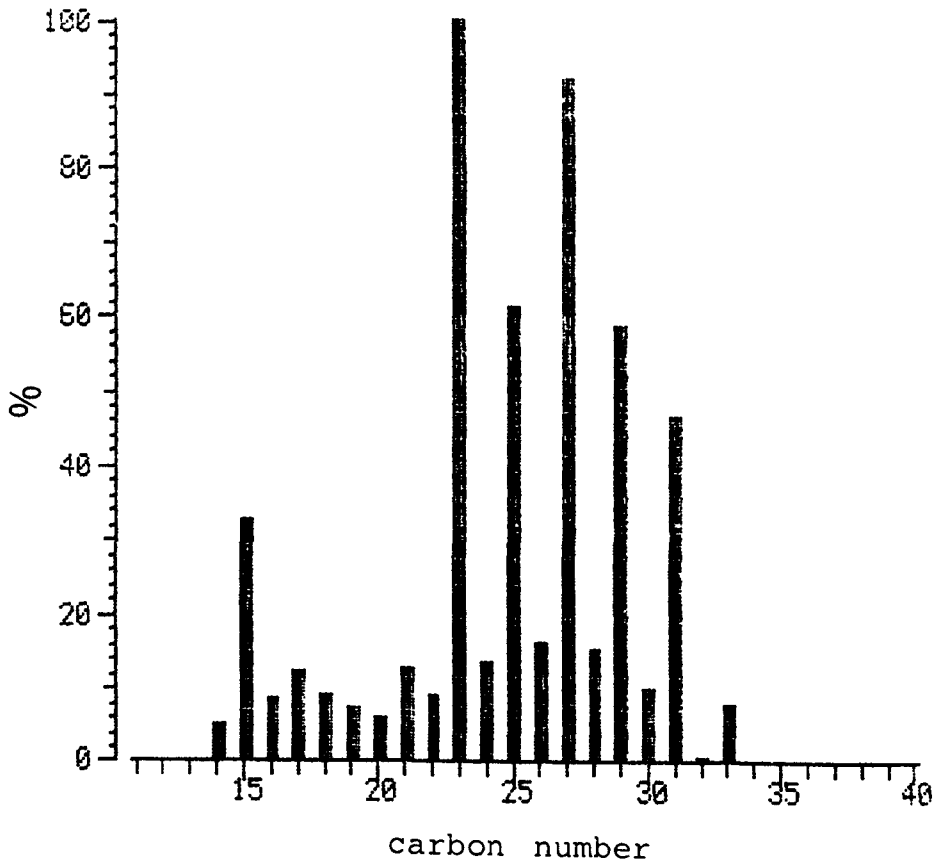


Fig.6.2/2. Distribution of n-alkanes in Messel oil shale.

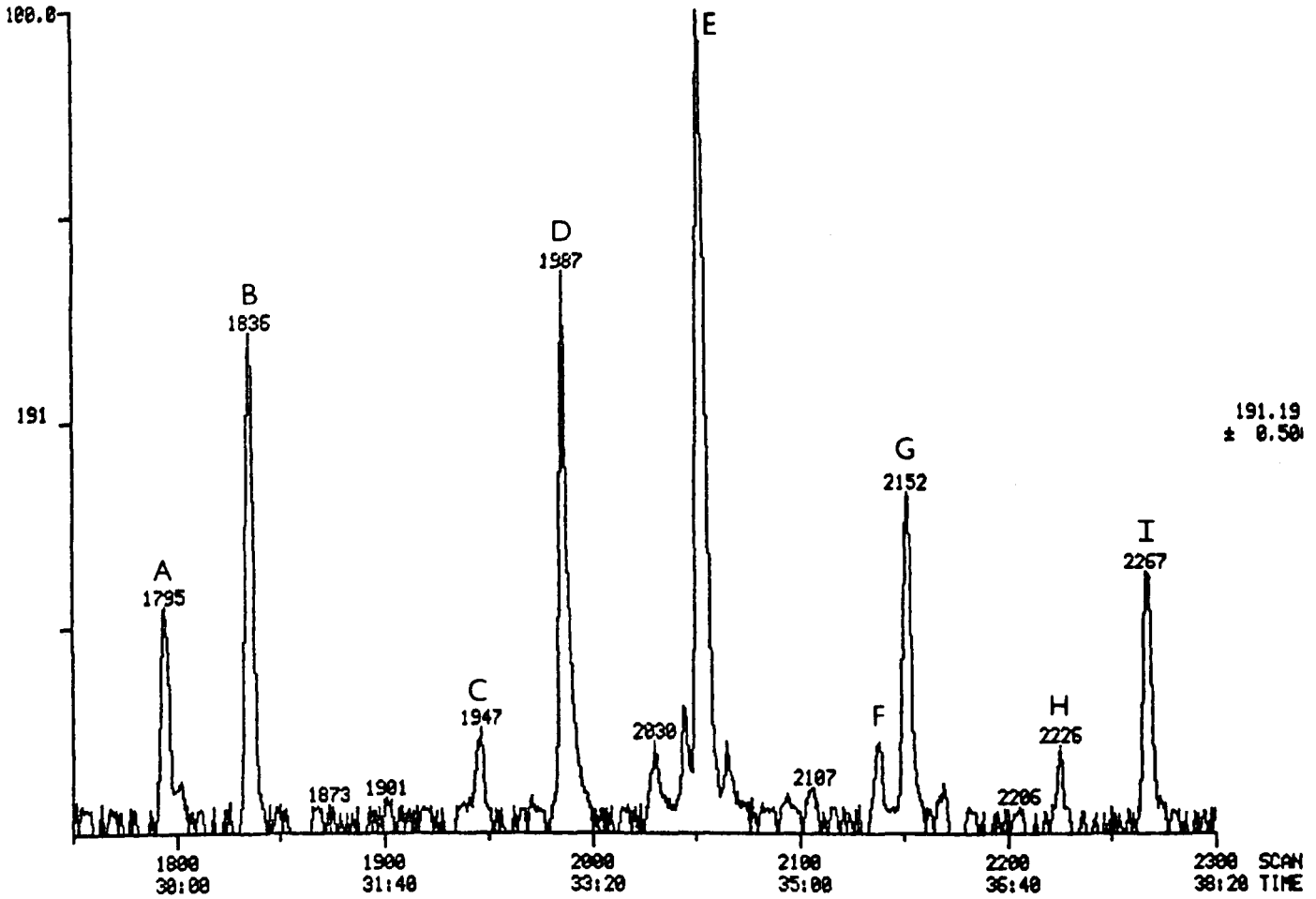


Fig. 6.2/3. m/z 191 Mass fragmentogram showing the distribution of aliphatic hopanoid hydrocarbons in Messel oil shale
Identities:-
A = 22,29,30-trisnorhop-17(21)-ene
B = 17β(H),21β(H)-22,29,30-trisnorhopane
C = norhop-17(21)-ene
D = hop-17(21)-ene
E = 17β(H),21β(H)-norhopane
F = 22R-17α(H),21β(H)-homohopane
G = 17β(H),21β(H)-hopane
H = homohop-30(31)-ene
I = 22R-17β(H),21β(H)-homohopane.
Identifications made by comparison of mass spectra and relative retention times with published data (e.g. Brooks et al., 1979; Wardroper, 1979). Operating conditions are given in Chapter 8.

a compound previously identified in Messel shale and proven to have structure LVII (Albrecht, 1969; Greiner et al., 1976).

6.2.ii Methyl Esters

A series of methyl esters was detected by GC-MS analysis of fraction T1; compounds ranged from n-C_{14} to n-C_{33}, with maxima at n-C_{25} and n-C_{29} (Fig. 6.2/4). One isoprenoid acid, C_{15}, was also recognised, present as the methyl ester.

6.2.iii Alcohols

a) Acyclic alcohols

A series of n-alkanols was detected ranging from C_{14} to C_{32}, maximising at C_{28} and having a high even carbon preference. One additional alcohol, eluting between the TMS ethers of n-C_{17} and n-C_{18} alcohols on an OV1 coated GC column, was detected. Its mass spectrum [m/z 341 (4%); 327 (1%); 266 (2%); 251 (2%); 131 (100%); 117 (10%); 75 (24%); 73 (44%)] was consistent with a C_{19} alcohol (TMS) (molecular weight 356 a.m.u.) with the hydroxyl group being situated on a dimethyl-substituted carbon or with an w-2 hydroxyl group:

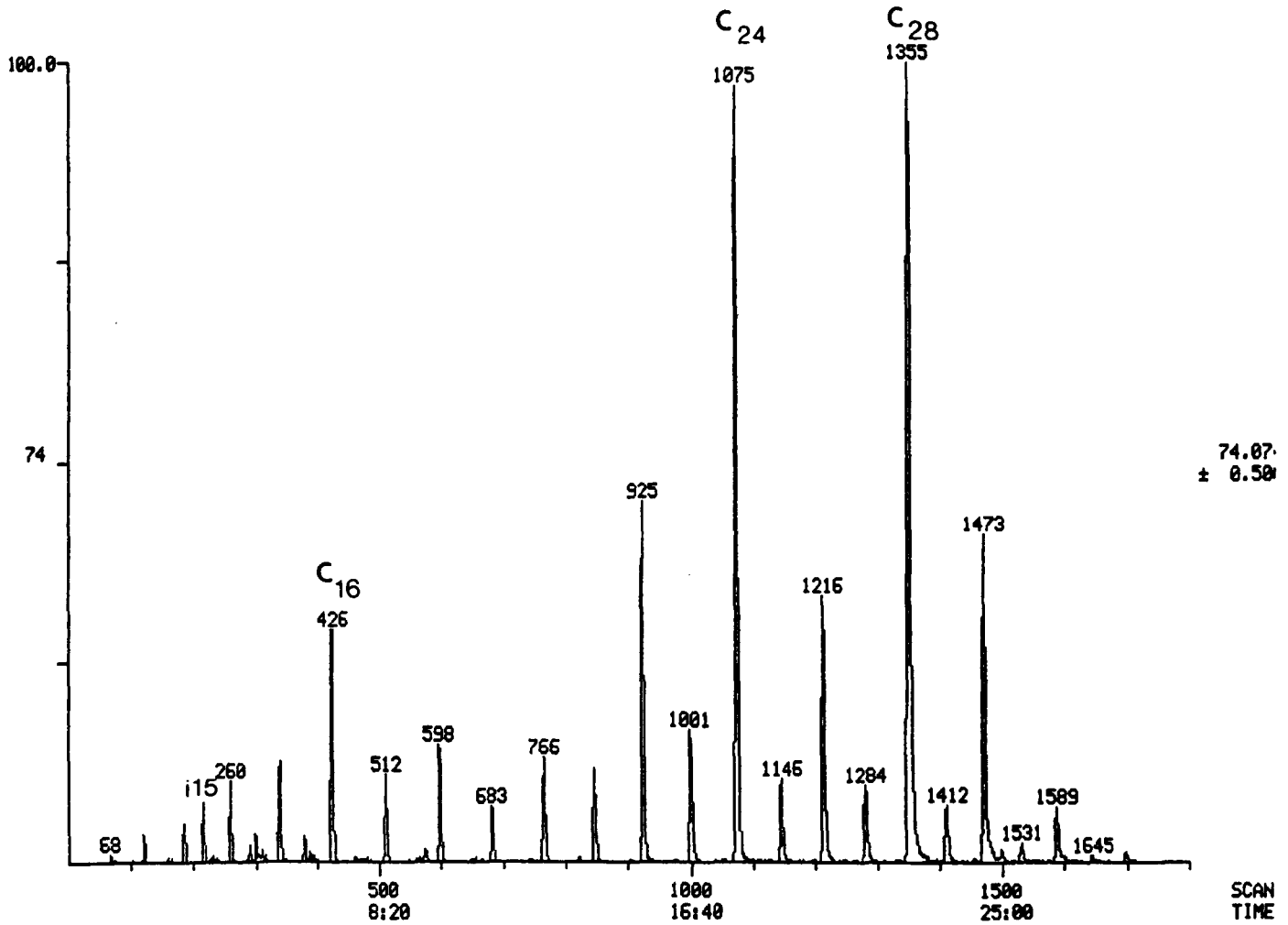
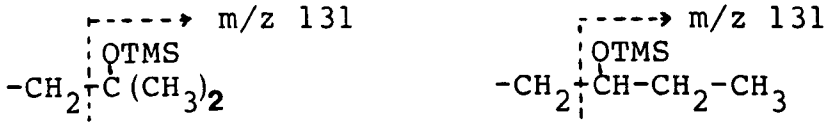


Fig. 6.2/4. m/z 74 Mass fragmentogram showing the distribution of methyl esters in Messel oil shale.
i15 = C_{15} isoprenoid acid present as methyl ester.
C_n = fatty acid containing n carbon atoms present as methyl ester



b) Cyclic alcohols

The major cyclic alcohols detected were a series of 4α-methylsterols, the distribution of which is shown in Fig. 6.2/5. The peak labelled M was found to consist of two coeluting compounds, one of which was n-C_{30} alkanol (TMS) and the other was identified as 4α-methyl,24-ethyl-5α(H)- cholest-22-en-3β-ol (XIIg) on the basis of its relative retention time, the general similarity of its mass spectrum with that of dinosterol and the prominent m/z 83 in its mass spectrum (indicating the presence of a Δ^{22},24-ethyl side-chain). The compound producing peak Q was tentatively identified as 4α,23-dimethyl,24-ethyl-5α(H) cholest-28-en-3β-ol (TMS) (XIIn) by mass spectral interpretation (Fig. 6.2/6). Peak O was found to have a mass spectrum which was very similar to that of peak Q; most probably the two C_{31} sterols producing these peaks are stereoisomers, perhaps 24R and 24S. The major sterol, corresponding to peak N, had a mass spectrum consistent with a C_{30} 4α-methystanol. In the absence of unsaturation little information on the side-chain was provided by its mass spectrum, leaving a 23,24-dimethyl or a 24-ethyl side-chain as the most probable structures. Indirect evidence for a 23,24-dimethyl side-chain was provided when hydrogenation of the sterols was found to simultaneously diminish peak L (proven to have a 23,24-dimethyl Δ^{22} side-chain by cochromatography with standard dinosterol) and enhance peak N

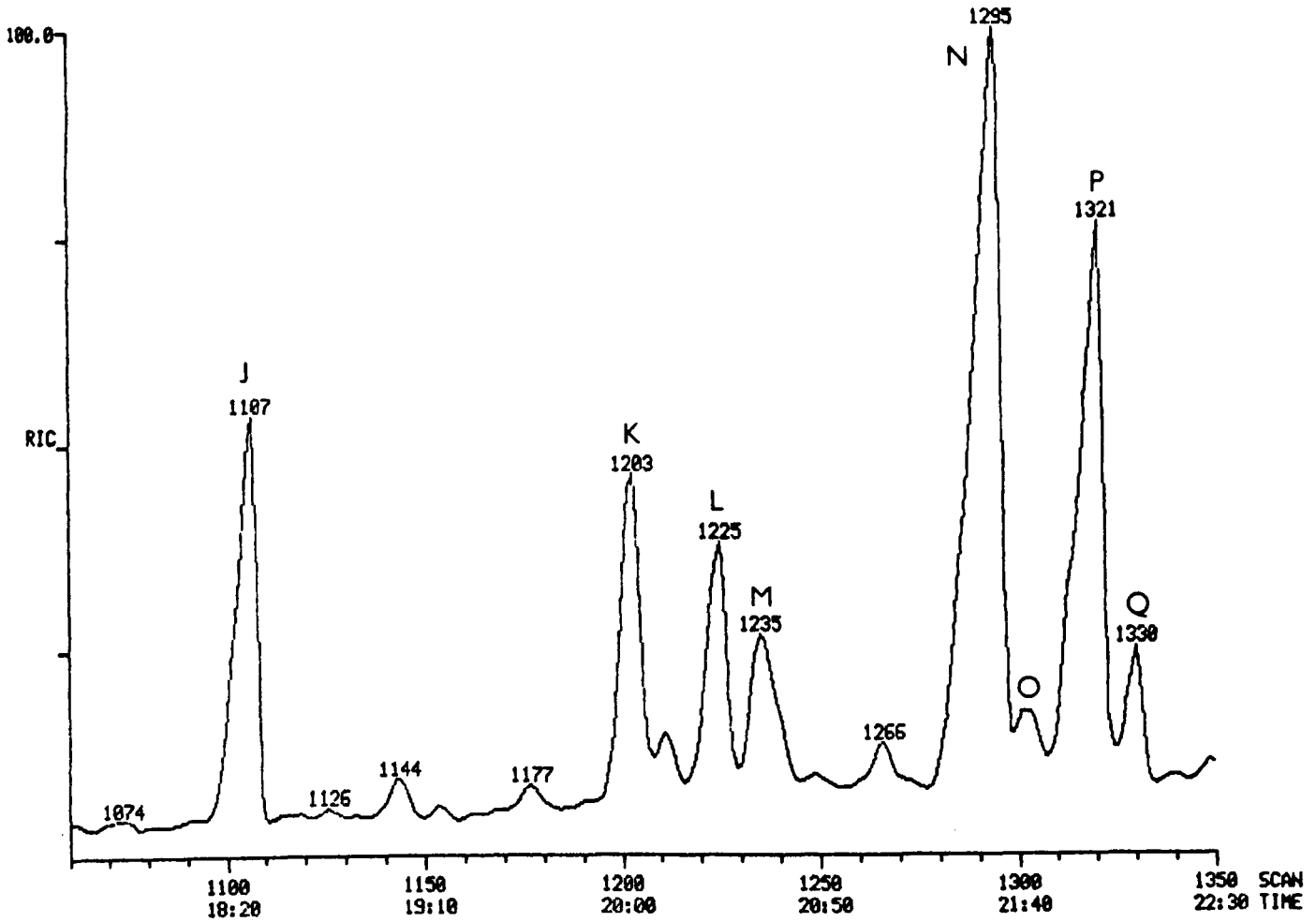
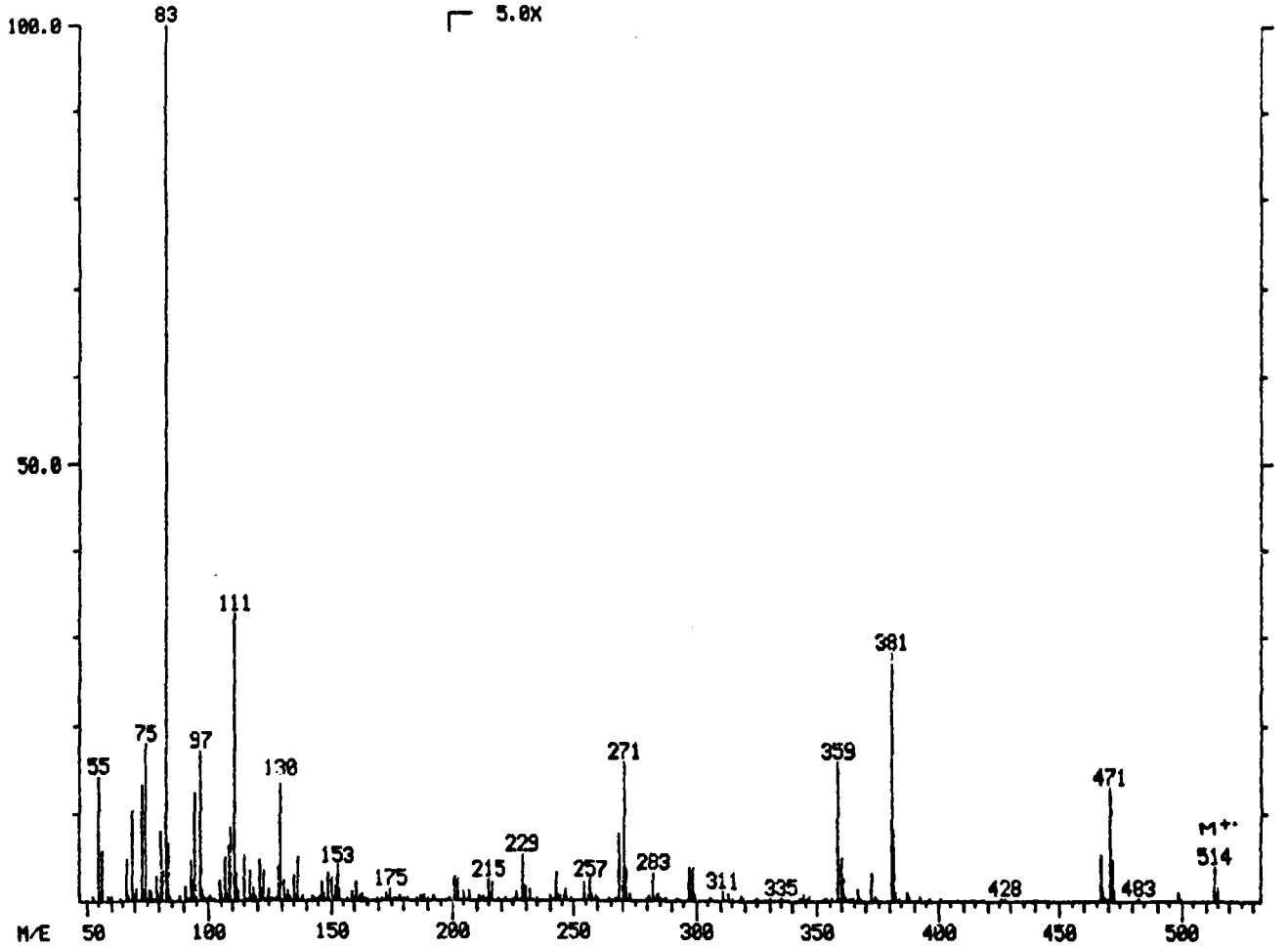


Fig. 6.2/5. Partial RIC showing distribution of sterols in Messel oil shale.
Identities:-
J = 4α-methyl-5α(H)-cholestan-3β-ol (XIIa)
K = 4α,24-dimethyl-5α(H)-cholestan-3β-ol (XIIe)
L = 4α,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol[a] (XIIi)
M = n-C_{30} alkanol + 4α-methyl,24-ethyl-5α(H)-cholest-22-en-3β-ol (XIIg)
N = 4α,23R,24R-trimethyl-5α(H)-cholestan-3β-ol (XIIj) + 4α,-methyl,24-ethyl-5α(H)-cholestan-3β-ol[b] (XIIh)
O = 4α,23-dimethyl,24-ethyl-5α(H)-cholest-28-en-3β-ol[c] (XIIn)
P = isoarborinol (XXIII)
Q = 4α,23-dimethyl,24-ethyl-5α(H)-cholest-28-en-3β-ol[c] (XIIn)

Compounds identified by comparison of mass spectra and relative retention times with published data unless otherwise stated.
(a) Identified by cochromatography with a standard.
(b) See text. (c) Identifications based on mass spectral interpretation.
All compounds present as TMS ethers. Operating conditions are described in Chapter 8.



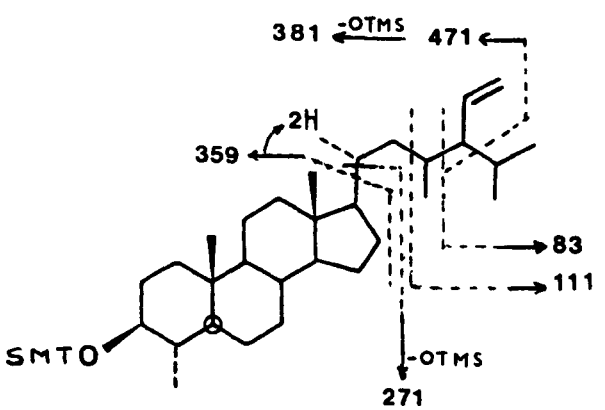
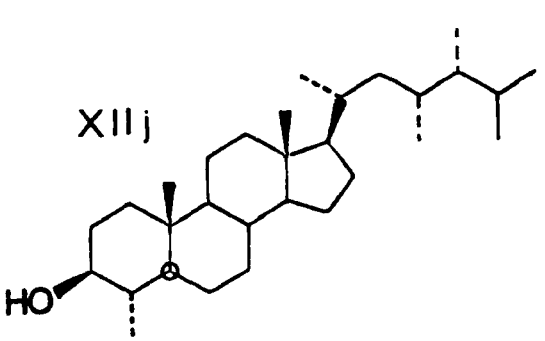
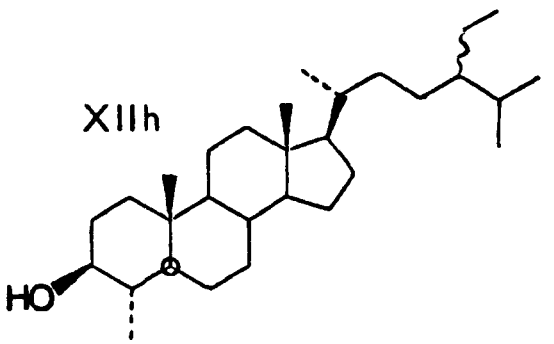


Fig. 6.2/6. Mass spectrum of peak labelled Q in Fig. 6.2/5.

(Fig. 6.2/7). Direct evidence was provided by cochromatography with a DSDP alcohol fraction (75-532-5-2 NIII), known to contain both 4α,23S,24R-trimethyl-5α(H)-cholest-3β-ol and 4α,23R,24R-trimethyl-5α(H)-cholest-3β-ol (Dr. S.C. Brassell, personal communication), coelution occurring with the latter (Fig. 6.2/8). In order to determine unambiguously the structure of the sterol producing peak N standards (kindly donated by Prof. C. Djerassi, Stanford Univ., California) of 4α,23R,24R-trimethyl-5α(H)-cholestan-3β-ol (XIIj) (dinostanol), 4α-methyl,24R-ethyl-5α(H)-cholest-3β-ol and 4α-methyl,24S-ethyl-5α(H)-cholestan-3β-ol (XIIh) were obtained for cochromatography.





Analysis of the standards, as TMS ethers or as free sterols, on capillary columns (25m and 50m) wall-coated with OV1 liquid phase and on a capillary column (50m) wall-coated with CPSil 5 liquid phase, fail to produce any separation. Published data (Withers, 1983) indicated that dinostanol could be resolved from the two 4α-methyl,24-ethylstanols (which coelute) on 3% SP2250 at 260 C as the free sterols, relative retention times of 1.81 and 1.83 respectively being reported, where cholesterol was 1.00. Thus, the three stanols were analysed on a capillary column (30m x 0.32mm) wall-coated with SP2250 (0.2um film thickness) programmed from 50 to 230° C (the maximum temperature for the

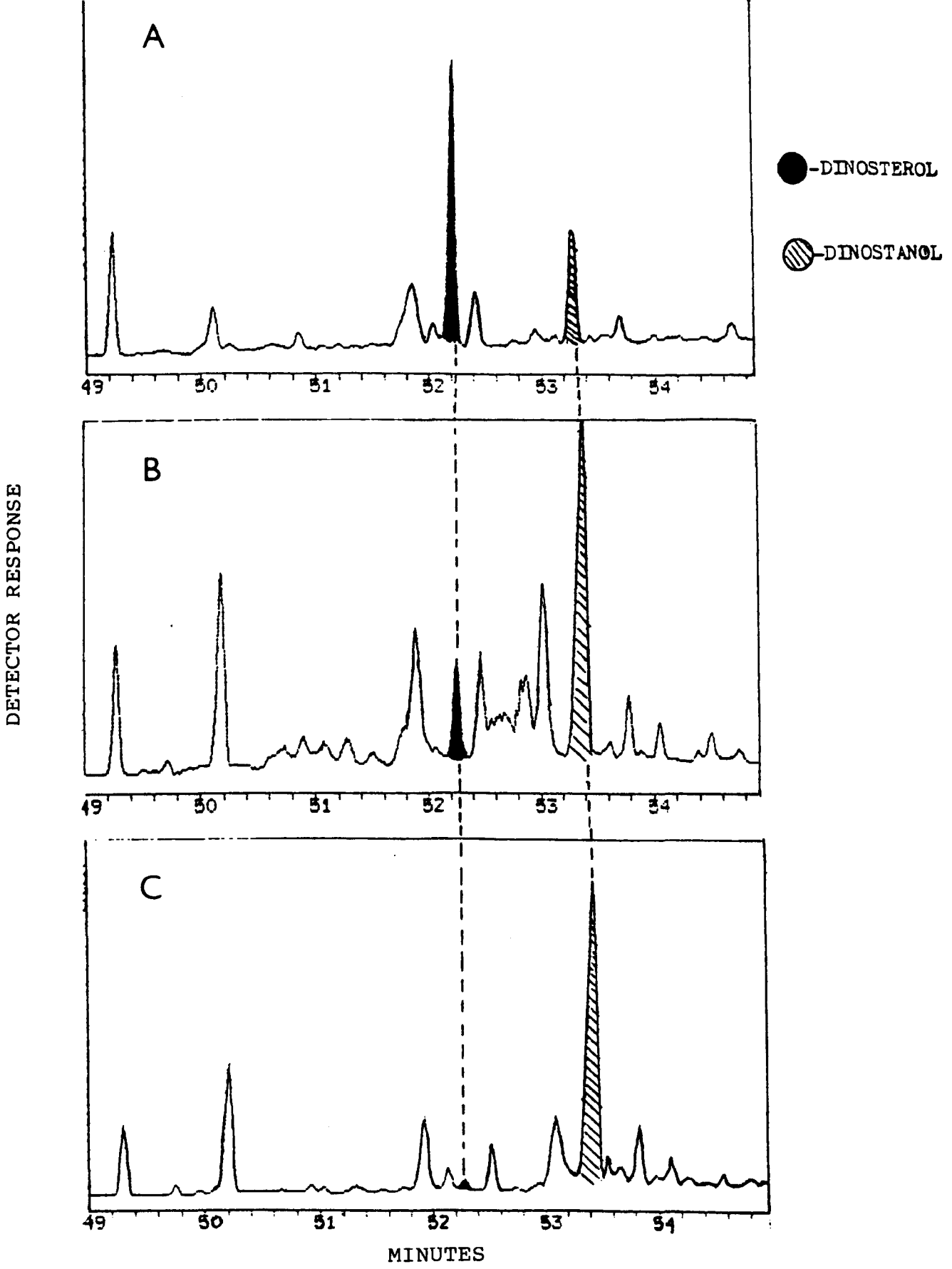


Fig. 6.2/7. Cochromatography of Messel alcohols with standard dinosterol and hydrogenation of Messel alcohols.
(A) Partial GC of Messel alcohols + dinosterol.
(B) Partial GC of Messel alcohols.
(C) Partial GC of Messel alcohols after hydrogenation.
All alcohols present as TMS ethers. Chromatographic and hydrogenation conditions are given in Chapter 8.

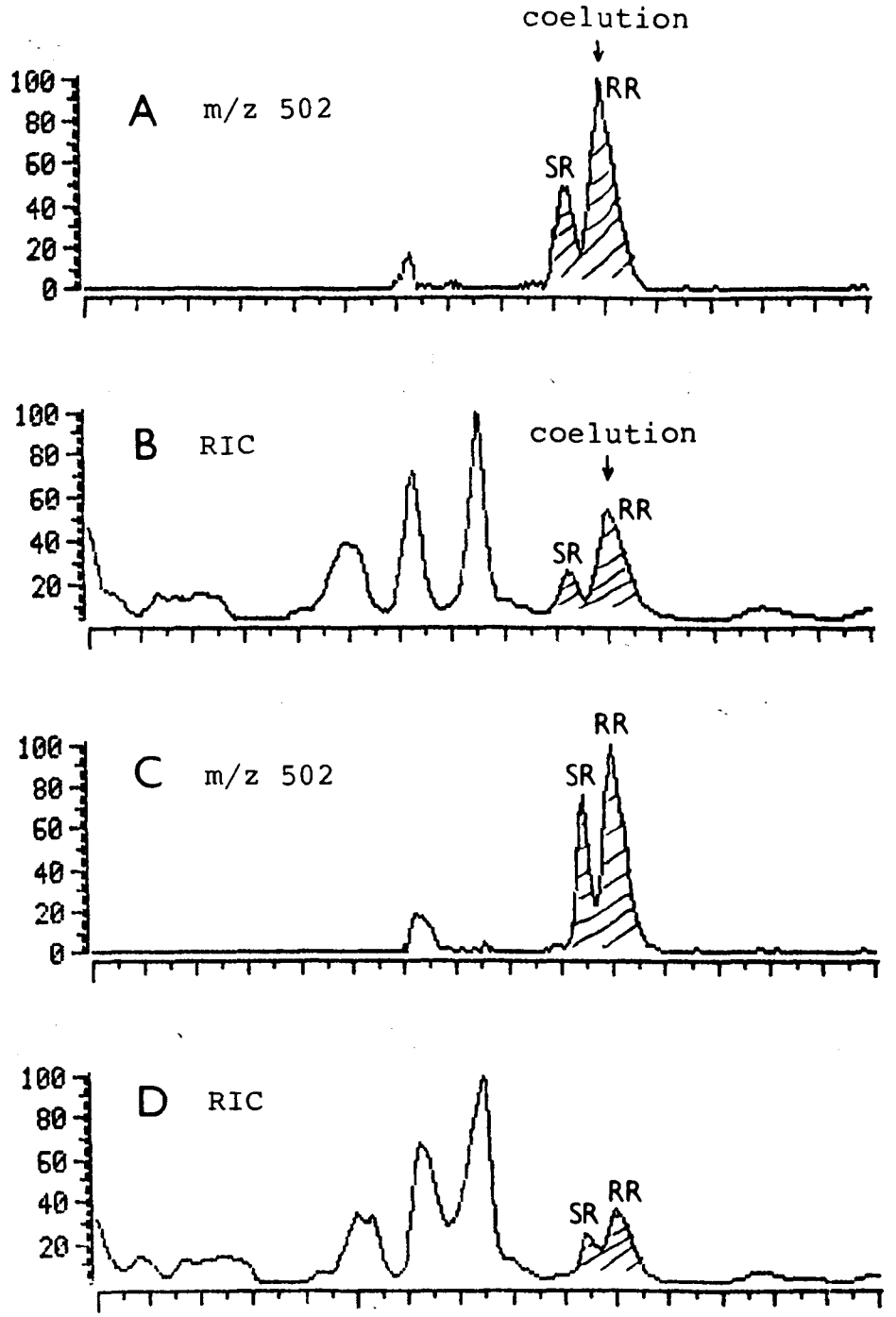


Fig. 6.2/8. GC-MS cochromatography of Messel alcohols with a DSDP alcohol fraction containing 4α,23S,24R-trimethyl-5α(H)-cholestan-3β-ol and 4α,23R,24R-trimethyl-5α(H)-cholestan-3β-ol. (Dr. S.C.Brassell, personal communication)

(A) m/z 502 mass fragmentogram (molecular ion for C_{30} stanol TMS) of Messel alcohols + DSDP alcohols.
(B) RIC of Messel alcohols + DSDP alcohols.
(C) m/z 502 mass fragmentogram of DSDP alcohols.
(D) RIC of DSDP alcohols.
All alcohols present as TMS ethers. Standard GC-MS conditions were employed (see Chapter 8) using a 15 m OV1 flexsil column.

column) at 4 min^{-1} and then held at isothermal. Using hydrogen as carrier gas, two peaks were produced eluting after *ca.* 80 and 90 minutes into the isothermal period respectively, the first eluting corresponding to dinostanol and the second to the two 4α-methyl,24-ethyl stereoisomers. Cochromatography under the above conditions of the Messel sterols with dinostanol and with 4α-methyl,24R-ethyl-5α(H)-cholestan-3β-ol produced enhancement in two peaks having similar areas. The major sterol in the sample of Messel shale studied was, therefore, identified as a mixture of 4α,23R,24R-trimethyl-5α(H)-cholestan-3β-ol and 4α-methyl,24-ethyl-5α(H)-cholestan-3β-ol (see, however, 6.2.v.b).

Two triterpenoid alcohols were identified, isoarborinol (peak P, Fig. 6.2/5) and a small amount of a 17β(H),21β(H)- hopan-29-ol.

c) Alkan-diols and alkan-keto-ols

A series of alkan-diols and alkan-keto-ols, eluting after the 4α-methylsterols in the same TLC fraction, was detected. The distribution of these compounds is shown in Fig. 6.2/9 and their identifications are given in Table 6.2/2. C_{30} to C_{32} compounds (R, S, T) were identified by comparison of mass spectra with published data (De Leeuw *et al.*, 1981); compound V was identified by mass spectral with those of compounds R, T and U (Fig. 6.2/10). Compound X was tentatively identified as a C_{34}-15-ol by mass spectral interpretation; compound A′ had a mass spectrum consistent with a $C_{33:1}$ keto-ol and compound W was an unknown alkan-diol (Fig. 6.2/11).

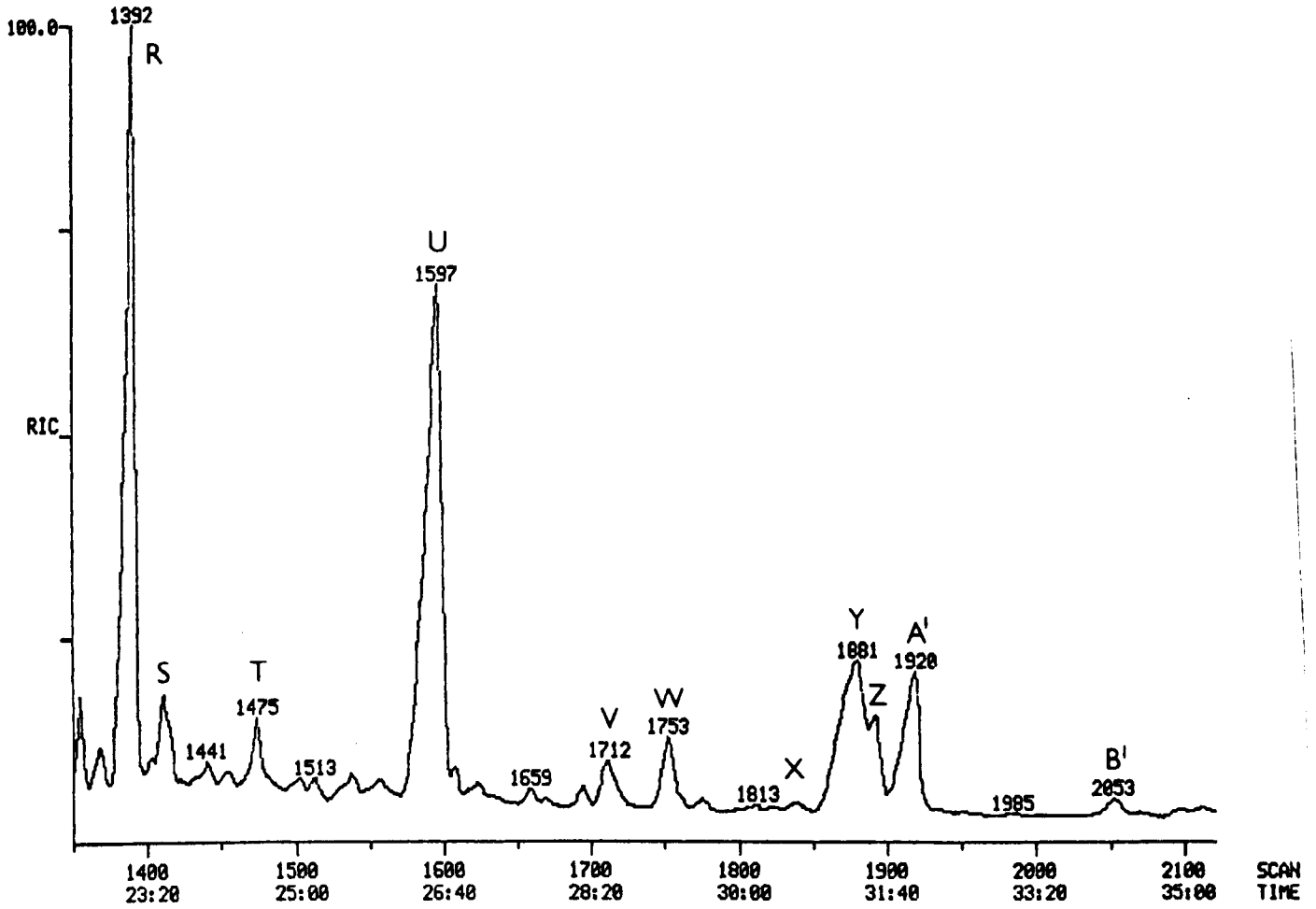


Fig. 6.2/2. Partial RIC showing the distribution of alkandiols and hydroxyalkanones in Messel oil shale.
Identifications are given in Table 6.2/5. For conditions see Fig. 6.2/5.

Table 6.2/2 Identification of alkandiols and hydroxyalkanones in Messel oil shale

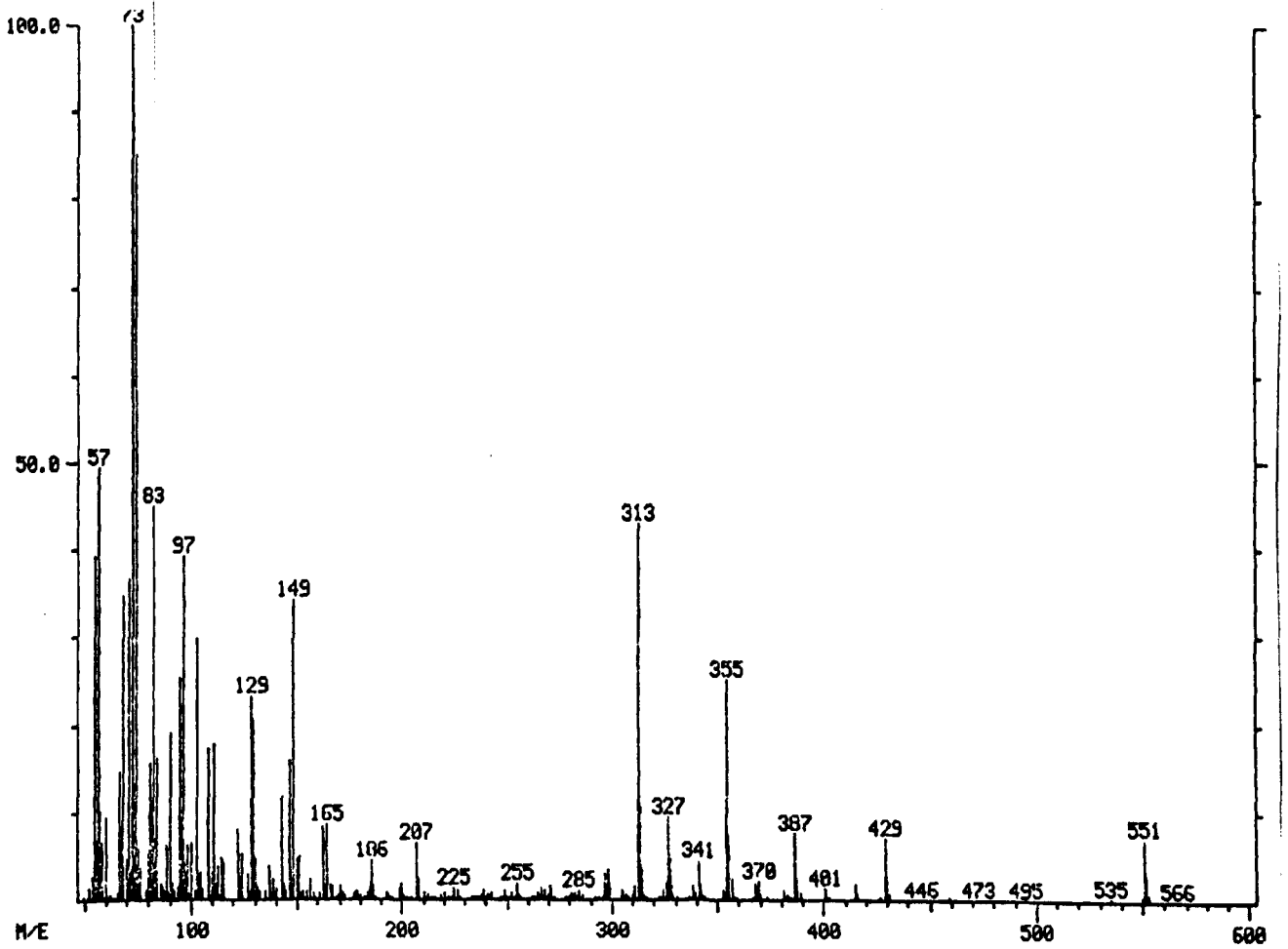
Peak[a]	Diagnostic mass spectral ions (m/z)[b]	Identification[c]
R	$M^{\cdot+}$ 524; M-15; 328; 326; 313; 311; 130; 75.	C_{30} alkan-15-one-1-ol
	387; 313; 130; 75; 73.	C_{30} alkan-1,15-diol
S	523; 75.	n-C_{32} alkanol
	525; 509; 117; 75.	C_{28}(w-1)-hydroxy methyl ester
T	$M^{\cdot+}$ 538; M-15; 340; 328; 325; 313; 130.	C_{31} alkan-15-one-1-ol
	387; 327; 130; 75; 73.	C_{31} alkan-1,15-diol
U	$M^{\cdot+}$ 552, M-15; 354; 339; 328; 313; 130.	C_{32} alkan-15-one-1-ol
	387; 341; 130; 75; 73.	C_{32} alkan-1,15-diol
V[d]	$M^{\cdot+}$ 566; M-15; 368, 353; 328; 313; 130.	C_{33} alkan-15-one-1-ol
	429; 313; 130; 75; 73.	C_{33} alkan-1,18-diol
	387; 355; 130; 75; 73.	C_{33} alkan-1,15-diol
	401; 327; 130; 75; 73.	C_{33} alkan-1,17-diol
	415; 341; 130; 75; 73.	C_{33} alkan-1,16-diol
W	See Fig. 6.2/11	Unknown
X	See Fig. 6.2/11	C_{34} alkan-15-ol?
Y	415; 341; 75; 73.	C_{34} alkan-1,17-diol
	387; 369; 75; 73.	C_{34} alkan-1,15-diol
Z	$M^{\cdot+}$ 580; M-15; 356; 354; 341; 339; 130.	C_{34} alkan-17-one-1-ol
	$M^{\cdot+}$ 580; M-15; 382; 367; 328; 313; 130.	C_{34} alkan-15-one-1-ol
A′	See Fig. 6.2/11	$C_{33:1}$ keto-ol?
B′	415; 355; 75; 73.	C_{35} alkan-1-,17-diol
	429; 341; 75; 73.	C_{35} alkan-1,18-diol
	443; 327; 75; 73.	C_{35} alkan-1,19,diol

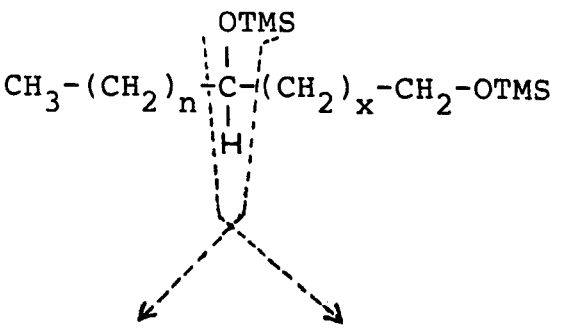
(a) Refers to Fig. 6.2/9

(b) Identifications based on mass spectral interpretation by comparison with published data (De Leeuw et al., 1981)

(c) Where positional isomers of the same compound coelute they are given in order of relative abundance. All hydroxy groups were present as TMS esters.

(d) See Fig. 6.2/10.





C_{33} 1,18-diol n = 14 m/z 313 x = 16 m/z 429
C_{33} 1,17-diol n = 15 m/z 327 x = 15 m/z 415
C_{33} 1,16-diol n = 16 m/z 341 x = 14 m/z 401
C_{33} 1,15-diol n = 17 m/z 355 x = 13 m/z 387

Fig. 6.2/10. Mass spectrum of peak labelled V in Fig. 6.2/9. Ions arising from a coeluting C_{33} alkan-15-one-1-ol are also present (see Table 6.2/2).

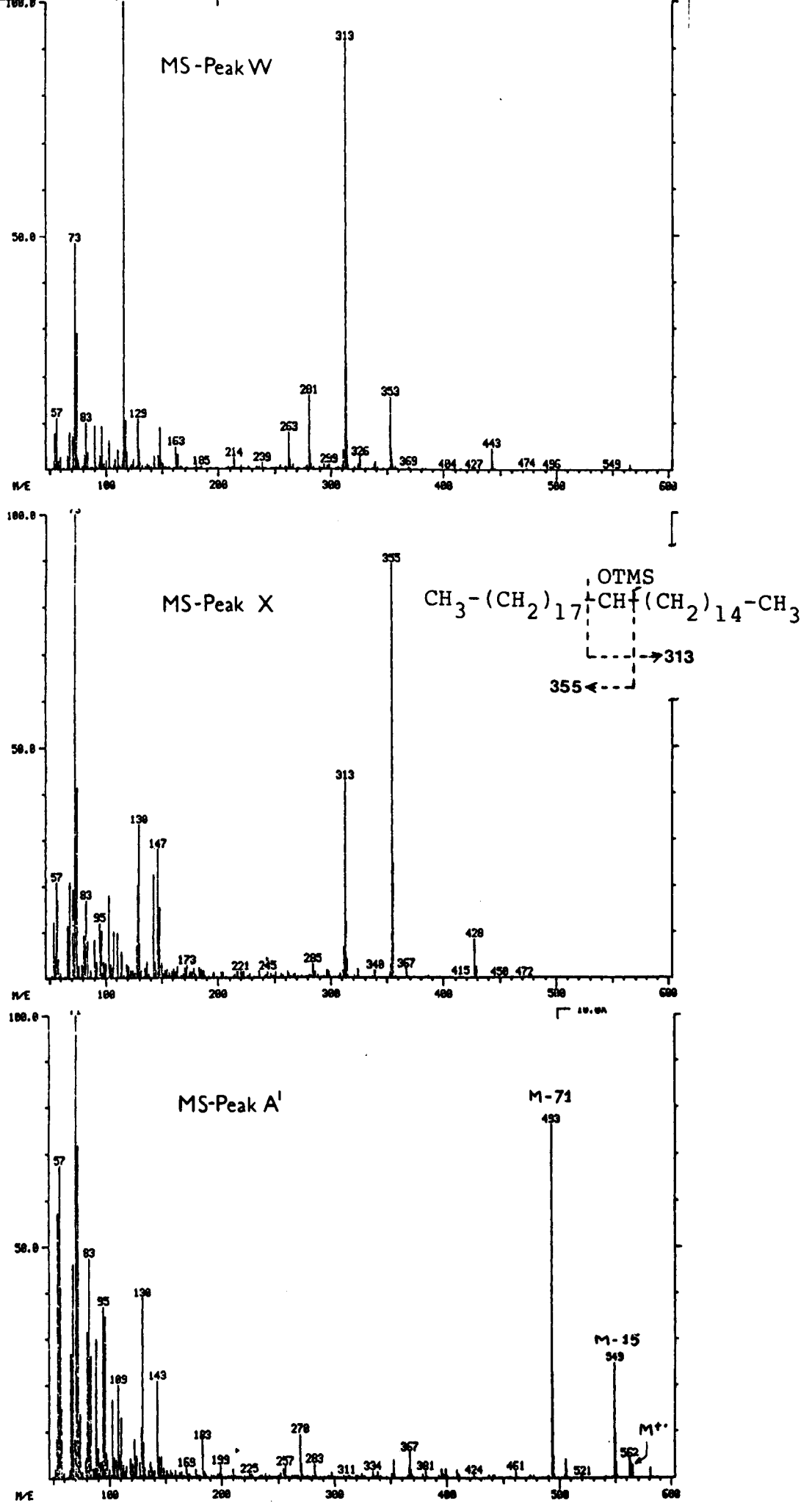


Fig. 6.2/11. Mass spectra of peaks labelled W,X and A´ in Fig. 6.2/9.

6.2.iv Ketones

a) Mid-chain ketones

A series of mid-chain ketones were identified over the range C_{27} to C_{34}, having an odd over even carbon number predominance and with positional isomers coeluting in the GC. The relative abundances and molecular compositions of these compounds are given in Table 6.2/3.

b) Alkan-2-ones

Straight chain alkan-2-ones were detected over the range C_{13} to C_{33}, maximising at C_{13} and C_{29} and having a high CPI (Fig. 6.2/12). The major component, however, was 6,10,14-trimethylpentadecan-2-one.

c) Cyclic ketones

Three series of cyclic ketones were detected, 4α-methylsteroidal ketones; hopanoid ketones and other pentacyclic triterpenoid ketones. Identities and relative abundances are shown in Table 6.2/4, the distribution of the hopanoid ketones is shown by an m/z 191 mass fragmentogram in Fig. 6.2/13.

6.2.v Interconversion of compound classes

a) Sterol ⇄ Sterone interconversions

Oxidation of an aliquot of the alcohol fraction resulted in a

Table 6.2/3 Molecular composition and relative abundances of mid-chain ketones from Messel oil shale[a]

Chain length[b]			% of chain length	% total ketones
Total	R_1	R_2		
27	13	13	55	5.4
	11	15	45	
28	13	14	81	4.1
	12	15	19	
29	13	15	89	27.0
	11	17	11	
30	14	15	71	11.6
	13	16	19	
	12	17	5	
	13	18	5	
31	15	15	62	25.6
	13	17	31	
	14	18	4	
	11	19	3	
32	15	16	78	16.8
	14	17	12	
	13	18	7	
	12	19	3	
33	15	17	75	6.9
	13	19	16	
	11	21	7	
	14	18	2	
34	15	18	61	2.5
	16	17	39	

(a) Identities made by comparison of mass spectra with published data (Wollrab, 1969).

(b) Refers to $R_1 - \underset{\underset{O}{\|}}{C} - R_2$

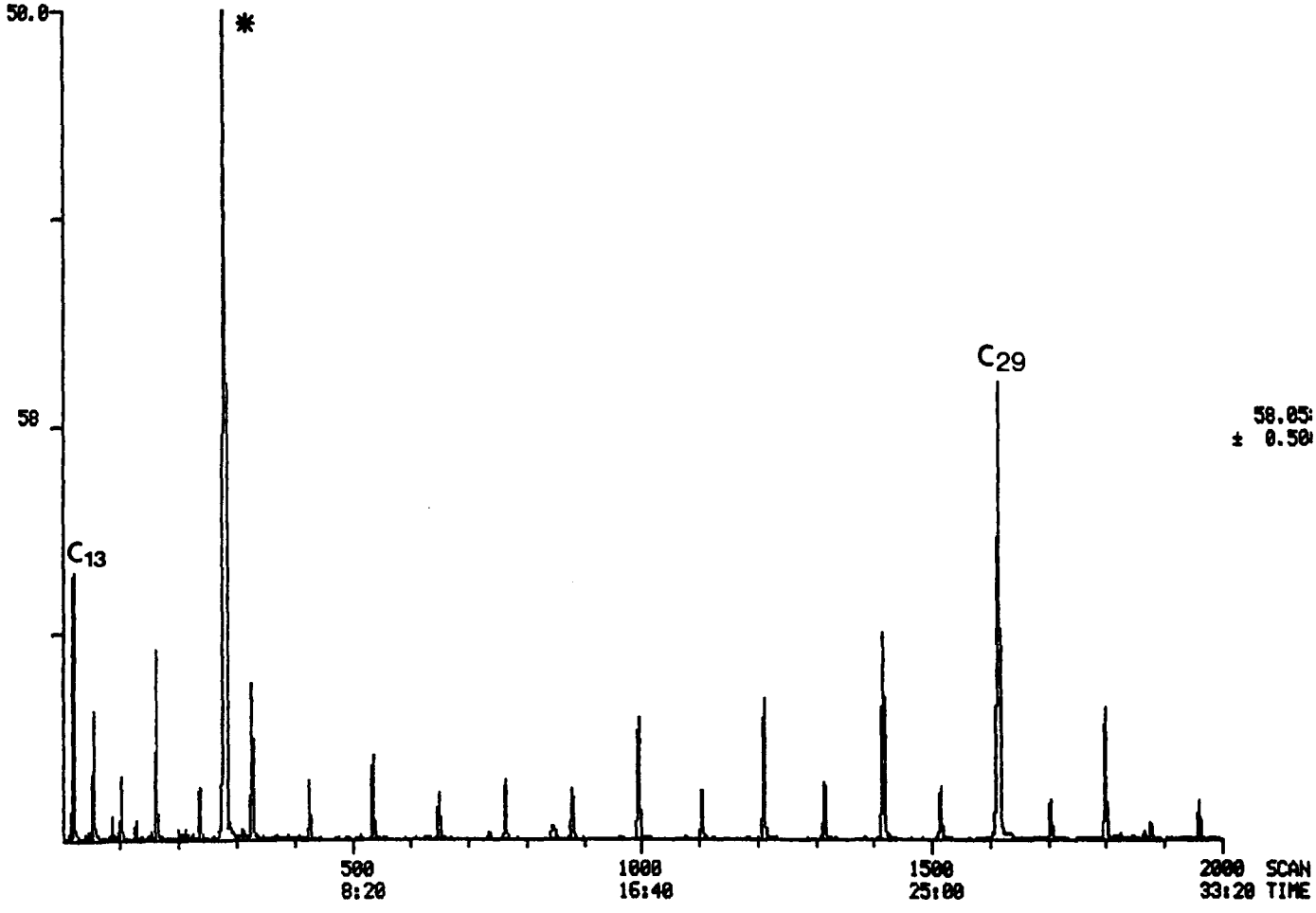


Fig. 6.2/12. m/z 58 Mass fragmentogram showing the distribution of alkan-2-ones in Messel oil shale.
* = 6,10,14-trimethylpentadecan-2-one.
Operating conditions are given in Chapter 8

Table 6.2/4 Distribution of cyclic ketones in Messel oil shale

Compound[a]	C no.	Structure	Rel. abundance
4α-Methyl-5β(H)-cholestan-3-one	28		1.1
4α-Methyl-5α(H)-cholestan-3-one	28	XVI a	7.1
4α,24-Dimethyl-5α(H)-cholestan-3-one	29	XVI e	4.0
4α,23,24-Trimethyl-5α(H)-cholest-22-*en*-3-*one*	30	XVI i	4.5
4α-Methyl,24-ethyl-5α(H)-cholest-22-en-3-one	30	XVI g	2.0
4α,23,24-Trimethyl-5α(H)-cholest-3-one[b]	30	XVI j	13.3
4α,23-Dimethyl,24-ethyl-5α(H)-cholest-28(29)-en-3-one[c]	31	XVI n	0.5
4α,23-Dimethyl,24-ethyl-5α(H)-cholest-28(29)-en-3-one[c]	31	XVI n	1.6
22,29,30-Trisnorhopan-21-one	27	XL	2.1
30-Norhopan-22-one	29	XLI	2.7
Hopanone[d]	30	XLII	2.2
Hopanone[d]	30	XLII	0.7
Homohopanone[d]	31	XLIII	1.4
Homohopanone[d]	31	XLIII	0.9
Homohopanone[d]	31	XLIII	0.8
Bishomohopanone[d]	32		0.3
Bishomohopanone[d]	32		0.9
Bishomohopanone[d]	32		0.6
Arborinone	30	XXX	11.1
Friedelan-3-one	30	XXIX	2.2
			100

(a) Identifications made by comparison of mass spectra with those of standards unless otherwise stated.

(b) May also contain 4α-methyl,24-ethyl-5α(H)-cholestan-3-one.

(c) Tentative identification based on mass spectral interpretation, compounds may be 24R and 24S diastereomers.

(d) Hopanoid ketones of same carbon number present, presumably differing in stereochemistry. Representative structures for the C_{30} and C_{31} compounds are given in the appendix.

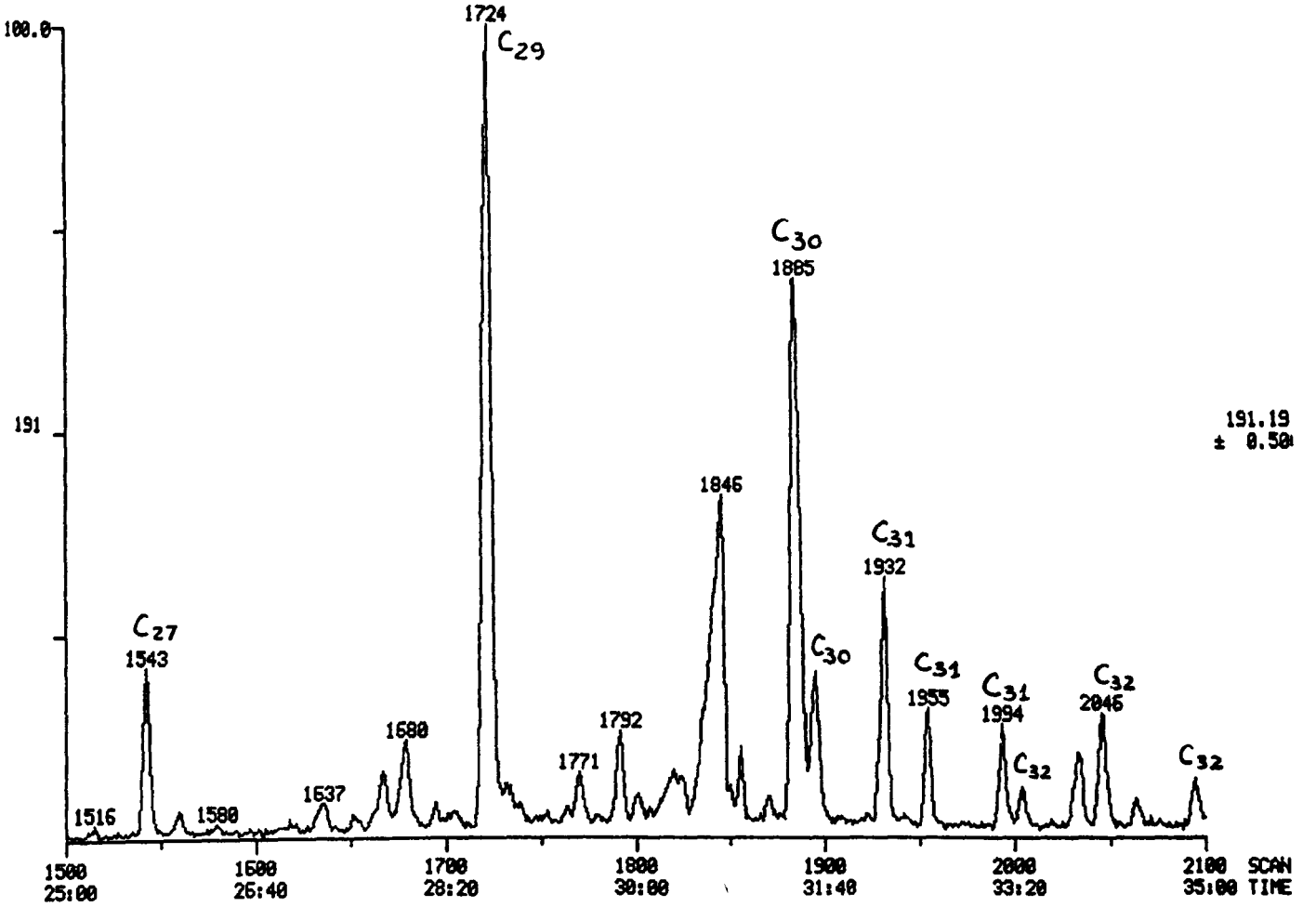


Fig. 6.2/13. m/z 191 Mass fragmentogram showing the distribution of hopanoid ketones in Messel oil shale. Operating conditions are given in Chapter 8.

ketone product. Analysis by GC and GC-MS revealed this to consist of arborinone (XXX) and a series of 4α-methylsteroidal ketones having the same distribution as the sedimentary ketones (Table 6.2/4). Further proof of the identical nature of the above compounds in the 'synthetic' ketone fraction and in the sedimentary ketone fraction was obtained by GC and GC-MS cochromatography using an OV1 coated column (Allen, 1984).

Reduction of an aliquot of the sedimentary ketone fraction with $NaBH_4$ in isopropanol resulted in an alcohol product in which steroidal ketones were specifically reduced to give sterols with a 3β-hydroxy stereochemistry. Analysis by GC and GC-MS (as TMS ethers) revealed the presence of a series of alkan-2-ols (originating from alkan-2-ones), isoarborinol and a series of 4α-methysterols. Alkan-2-ols did not appear in the sedimentary alcohols, but the 4α-methysterols and isoarborinol produced by reduction of sedimentary ketones had a very similar distribution to the sedimentary alcohols, and, furthermore, were shown to coelute in both GC and GC-MS analysis using OV1 liquid phase (Allen, 1984).

b) Conversion of 4α-methylstanols to 4α-methylsteroidal hydrocarbons

An aliquot of the sterols isolated from Messel oil shale gave, upon conversion of alcohols to alkanes (tosylation followed by treatment with $LiAlH_4$, see Chapter 8), a series of 4α-methylsteroidal hydrocarbons with the distribution shown in Fig. 6.2/14. Assignment of the stereochemistry at C-5 as 5α(H)

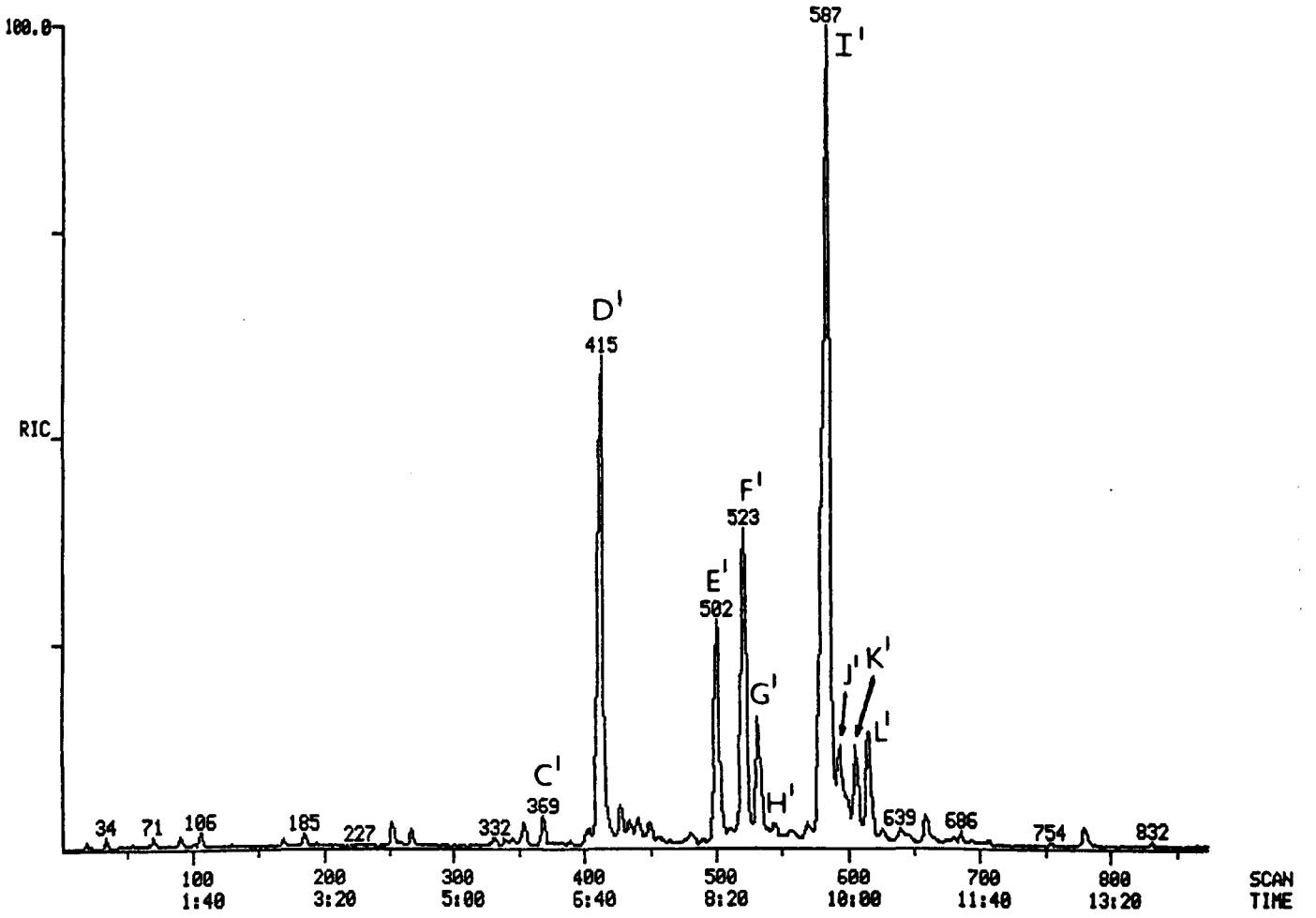


Fig. 6.2/14. Distribution of 4α-methylsteroidal hydrocarbons synthesised from Messel oil shale 4α-methylstanols (see Chapter 8).

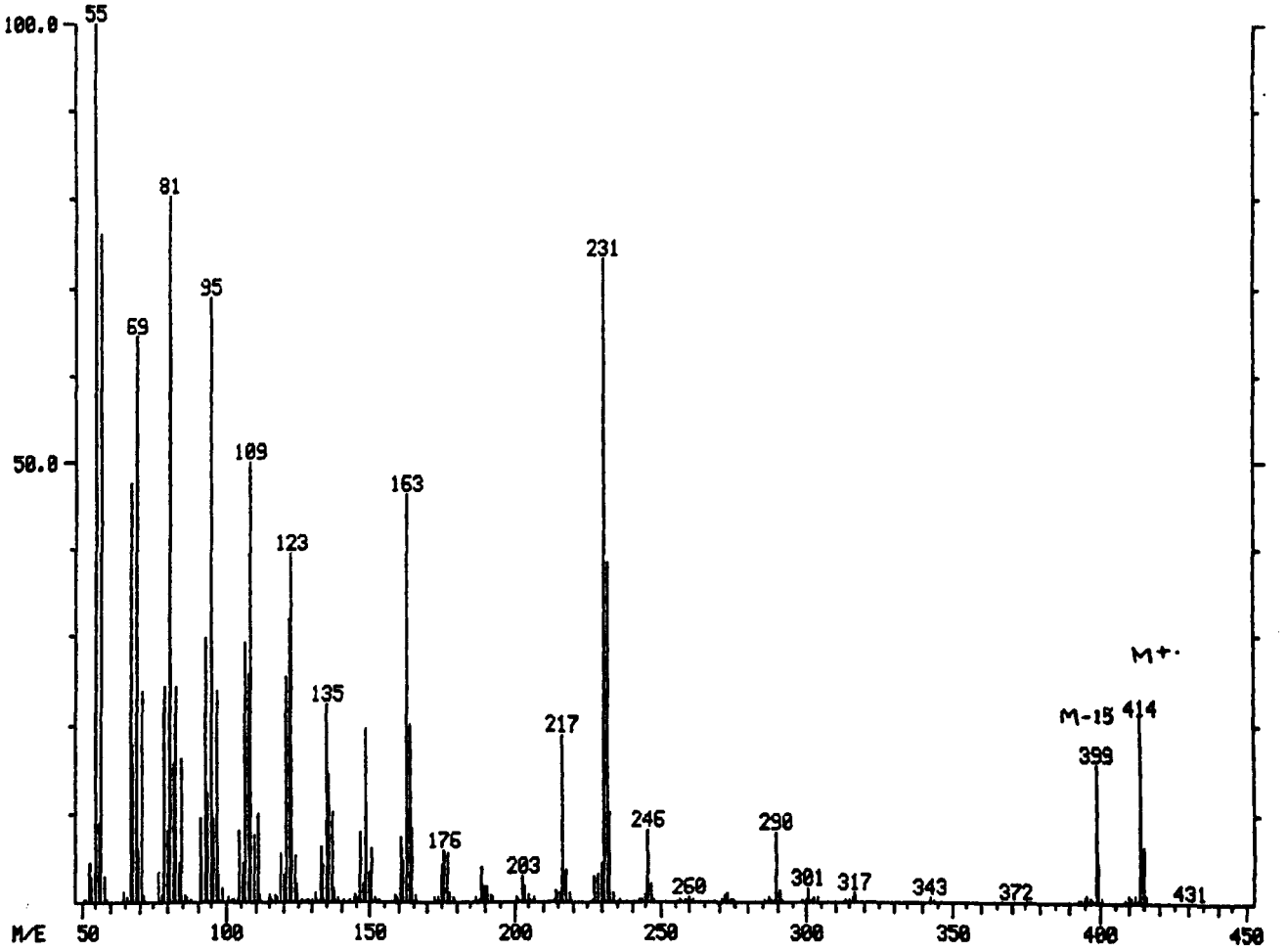
Identities[a]:-

C′ = 4α-methyl-5β(H)-cholestane
D′ = 4α-methyl-5α(H)-cholestane
E′ - 4α,24-dimethyl-5α(H)-cholestane
F′ = 4α,23,24-trimethyl-5α(H)-cholest-22-ene[b]
G′ = 4α-methyl,24-ethyl-5α(H)-cholest-22-ene[b]
H′ = 4α,23-dimethyl,24-ethyl-5β(H)-cholest-28-ene[b]
I′ = 4α,23,24-trimethyl-5α(H)-cholestane[c]
J′ = 4α,23-dimethyl,24-ethyl-5α(H)-cholest-28-ene[b]
K′ = 4α-methyl,24-ethyl-5α(H)-cholestane[c]
L′ = 4α,23-dimethyl,24-ethyl-5α(H)-cholest-28-ene[b,d]

(a) Identifications made by comparison of mass spectra with published data (Kimble et al., 1974b), unless otherwise stated.
(b) Identifications based on mass spectral interpretation.
(c) Identified on basis of relative retention order (see text). Mass spectrum of I′ is shown in Fig. 6.2/15.
(d) Mass spectrum of L′ is shown in Fig. 6.2/16.

Operating conditions are given in Chapter 8.

was made on the basis of m/z 163 > m/z 165 in the mass spectra (Kimble et al., 1974b). The mass spectrum of compound K′ was similar to that of compound I′, shown in Fig. 6.2/15, both being consistent with a C_{30} 4α-methylsterane. By analogy with the retention times of the corresponding stanols, I′, the first eluting C_{30} 4α-methylsterane, was assigned as 4α,23R,24R-trimethyl-5α(H)-cholestane and K′ as 4α-methyl, 24-ethyl-5α(H)-cholestane. The peak produced by I′ was very much larger than that produced by K′, implying that dinostanol is much more abundant than 4α-methyl,24-ethyl-5α(H)-cholestan-3β-ol in Messel oil shale. Differences in the side-chain would not be expected to cause differences in the rate of reaction at the hydroxyl group of the above two stanols. Compounds J′ and L′ had similar mass spectra and were tentatively identified as 24R and 24S stereoisomers of 4α,23-dimethyl,24-ethyl-5α(H)-cholest-28(29)-ene by mass spectral interpretation, comparing their mass spectra with that of compound F′; the mass spectrum of L′ is shown in Fig. 6.2/16. The minor compound H′ had a mass spectrum similar to those of compounds J′ and L′, except that m/z 165 was larger than m/z 163, suggesting that it might be the 5β(H)-isomer of one of them.



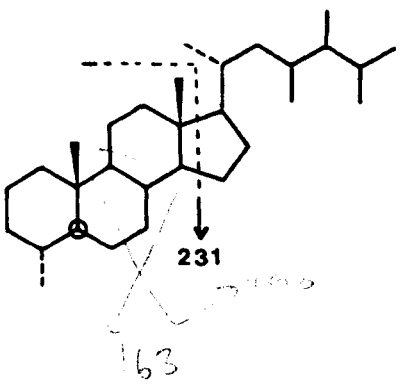
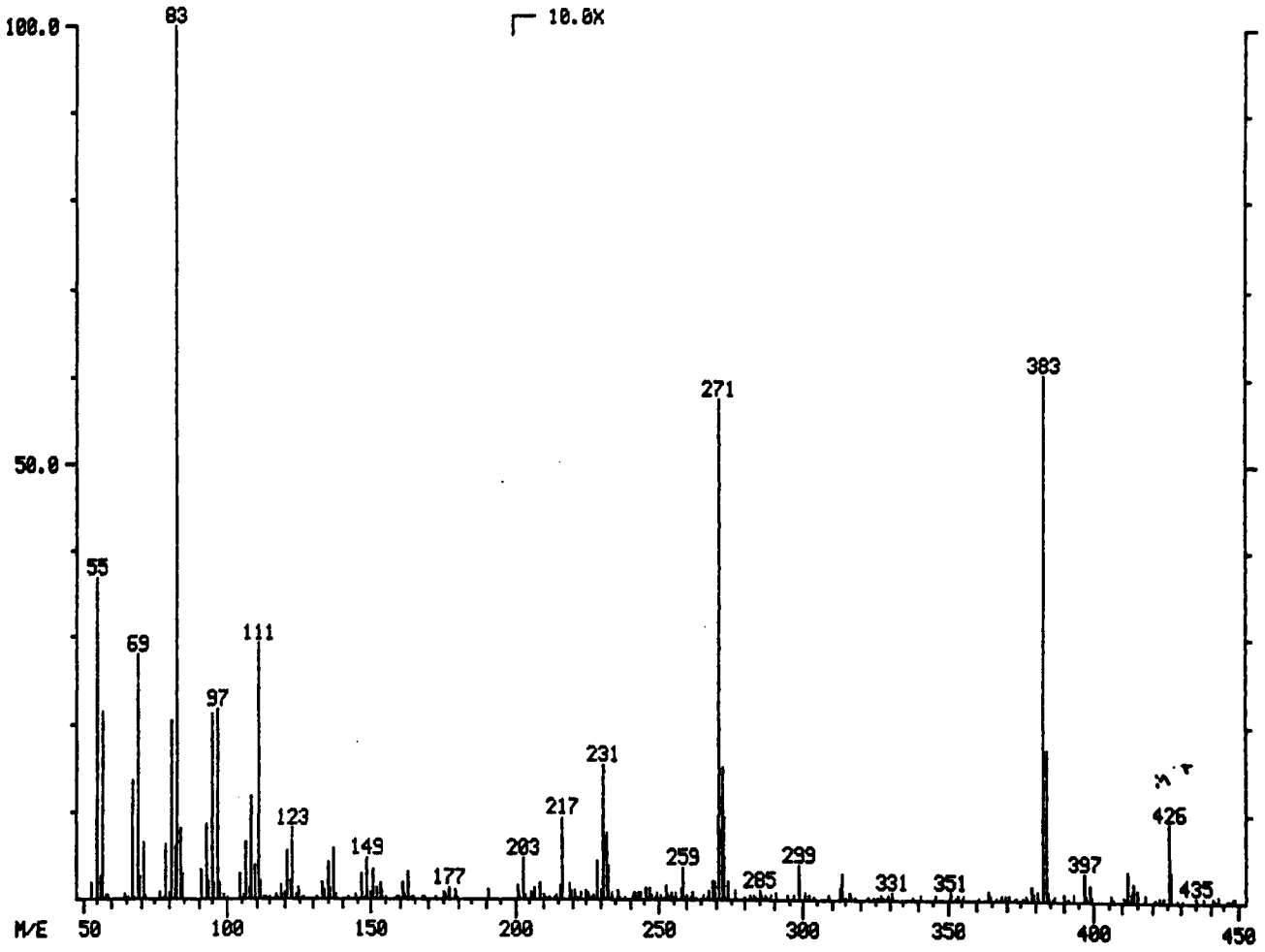


Fig. 6.2/15. Mass spectrum of peak labelled I′ in Fig. 6.2/14.



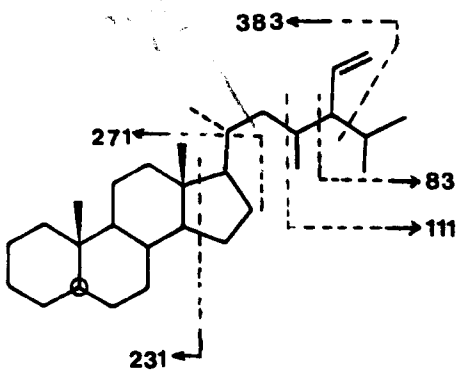


Fig. 6.2/16. Mass spectrum of peak labelled L′ in Fig. 6.2/14.

6.3 DISCUSSION

6.3.i Sources of lipids in Messel oil shale

The Messel oil shale is known to have been deposited in a shallow lacustrine environment, with higher plants contributing to the organic matter (Matthes, 1956, 1968; Sittler, 1968). A higher plant contribution can be recognised from the sedimentary lipids in the form of n-alkanes and n-alkanols with high CPI in the range C_{22} to C_{32} (Cranwell, 1973, 1976; Brooks et al., 1976; Cardoso et al., 1983). These distributions, however, cannot be used as quantitative measures of higher plant input to Messel as it is known that shorter chain homologues are preferentially degraded during diagenesis (Quirk, 1978; Cranwell, 1981a), and so the autochthonous contribution would be seriously underestimated.

The presence of 4α-methylsteroids in much greater abundance than 4-desmethylsteroids is unusual in sediments: we were unable to detect 4-desmethylsteroids, whilst Mattern et al. (1970) reported desmethylsterols to be present at a concentration 70 x less than that of the 4-methyl analogues. All of the sterols detected in the present study, with the exception of the novel

C_{31} compounds (XIIn) have been reported to occur in freshwater and/or marine dinoflagellates (Withers et al., 1978, 1979a,b; Kokke et al., 1981b, 1982; Robinson et al., 1984a; Chapter 3, this thesis). Furthermore, dinosterol and dinostanol, believed to be unique to dinoflagellates, have been shown to be present in Messel oil shale and it is, therefore, proposed that the 4α-methysterols present in the Messel oil shale have a dinoflagellate origin. Further work is required to determine the relative proportions of dinostanol (XIIj) and 4α-methyl,24-ethyl-5α(H)-cholestan-3β-ol (XIIh) in Messel oil shale. The 4α-methylsteroidal ketones are also proposed to have a dinoflagellate origin and are discussed further in the following section.

Desmethylsterols are major components of higher plants and also occur in dinoflagellates, and so their absence in Messel shale is puzzling. There is evidence to suggest that there are differences in the dehydration rate of sterols and 4-methylsterols to give the corresponding alkenes (Mackenzie et al., 1982), but if the low abundance of 4-desmethylsterols was due to preferential dehydration relative to 4-methylsterols, this should be reflected in the steroidal hydrocarbons. Although steroidal hydrocarbons were not present in the sample of Messel oil shale analysed in the present study, 4α-methylsteranes have been reported to greatly predominate over steranes in a previous study of Messel (Kimble et al., 1974a). Some dinoflagellates, such as the primitive species Glenodinium hallii, contain very low levels of 4-desmethylsterols (Alam et al., 1981), indeed, work described in this thesis has shown that the freshwater

dinoflagellate Woloszynskia coronata contains abundant 4α-methylsterols but no desmethylsterols (Chapter 3). It is possible that the dinoflagellates inhabiting the Messel lakes 50 million years ago contained low levels of desmethylsterols, but if that was the solution to the puzzle, then the lack of desmethylsterols in the sediment would imply a very small higher plant input. The results presented herein refer to extractable lipids of the Messel shale; possibly, analysis of the bound lipid fraction would help to determine why desmethylsterols are absent from the extractable lipid fractions.

Hopanoids form a major class of compounds amongst the extractable lipids of Messel shale. Current thinking assigns such compounds to a bacterial contribution to the sediment, originating either from direct bacterial input or from diagenesis of bacterial lipids such as polyhydroxybacteriohopanes (see Ourisson et al., 1979; Dastillung et al., 1980; Rohmer et al., 1980b). Hop-17(21)-ene (XXXI) and neohop-13(18)-ene may arise as isomerisation products of hop-22(29)-ene (XXXV) with hop-21-ene as an intermediate (Ensminger, 1977), however, the presence of only $\Delta^{17(21)}$ hopenes plus homohop-29(31)-ene (XXXVI) in Messel shale, makes a direct biological input of the hopenes more likely. Although hop-17(21)-ene has been reported to occur in ferns (Berti and Bottari, 1968) as well as in the photosynthetic bacterium Rhodomicrobium vanielli (Howard, 1980), the absence of other cyclic triterpenoids expected to be associated with a major angiosperm input, suggests that bacteria are the most important source of hopenes in the Messel oil shale. Isoarborinol (XXIII) and arborinone (XXX) are also suggested to represent bacterial

inputs to sediments, although such compounds are yet to be recognised in organisms (Brassell et al., 1983).

Alkan-diols and alkan-15-one -1-ols covering the range C_{28} to C_{32} have been reported to occur in Quaternary and Neogene Black Sea sediments, Quaternary sediments from the Middle America Trench and Guayamas Basin, and from a Quaternary Sapropel from the Eastern Mediterranean (De Leeuw et al., 1981; Brassell et al., 1981; Thomson et al., 1982; Smith et al., 1983). The recognition of alkan-diols and hydroxyalkanones in Messel shale, together with the detection of alkan-diols in Lake Kinneret sediment (Chapter 5, this thesis) and Priest Pot sediment (Chapter 4, this thesis), provides the first report of such compounds in lacustrine sediments; previously these compounds were proposed to have a marine source (Brassell et al., 1981; Smith et al., 1983). All of the sediments in which these diols and hydroxyalkanones have been recognised, including Messel oil shale, share several features in common: they are organic-rich deposits, formed under conditions of high biological productivity, with significant dinoflagellate inputs, and have experienced a relatively mild burial temperature history. Dinoflagellates, therefore, appear to be the primary contenders for a biological source of such compounds. The absence of alkan-diols or hydroxyalkanones in the extractable lipids of the dinoflagellates reported in Chapter 3 of this thesis, suggests that these compounds may exist in a bound form in dinoflagellates and be released by diagenesis in the sedimentary column (see Chapter 5). The occurrence of C_{33} to C_{35} diols and hydroxyalkanones in Messel oil shale extends the reported carbon

number range of such compounds.

Habermehl and Hundrieser (1983) have reported a series of ethyl esters in Messel shale. The extractable lipids of the sample of Messel shale used in the present study did not contain ethyl esters, but did contain methyl esters. As methanol was employed in the extraction procedure it is likely that the methyl esters are artifacts formed from fatty acids during extraction; alternatively, methyl esters were present as such in the sediment. Extraction with solvents other than methanol, e.g. THF, should resolve whether or not the methyl esters are artifacts. Both methyl and ethyl esters have been demonstrated to occur in certain dinoflagellates (Chapter 3, this thesis).

Mid-chain ketones have been previously reported to occur in a marine diatomaceous sediment (Boon and De Leeuw, 1979) and in 9000-14500 years old lacustrine sediments (Cranwell, 1984). C_{29} and C_{31} ketones having symmetrical structure, or with the carbonyl group slightly displaced from the centre, occur in higher plant waxes (Tulloch, 1976); biosynthesis occurs from long chain n-alkanes, by the introduction of oxygen to give a secondary alcohol which is then oxidised to the ketone (Kolattukudy, 1980). As the most widespread non-symmetrical ketone found in plants is the C_{29} alkan-10-one and the sedimentary ketones were also almost exclusively present having the oxygen attached to an even-numbered carbon atom, Cranwell (1984) proposed an origin from cuticular wax components. The odd carbon number mid-chain ketones present in Messel shale are also dominated by molecules having the oxygen attached to an even-numbered carbon atom (Table 6.2/3). Previously, the

mid-chain ketones observed in sediments have been exclusively odd carbon-number compounds, in the Messel, however, even carbon-number components are present. Mid-chain ketones in Messel shale were found over the range C_{27} to C_{34}, whereas Cranwell (1984) reported C_{29} to C_{39} compounds. The differences observed may be due to diagenesis in the much older Messel shale altering the original cuticular wax derived distribution, or else may reflect differences in source organisms.

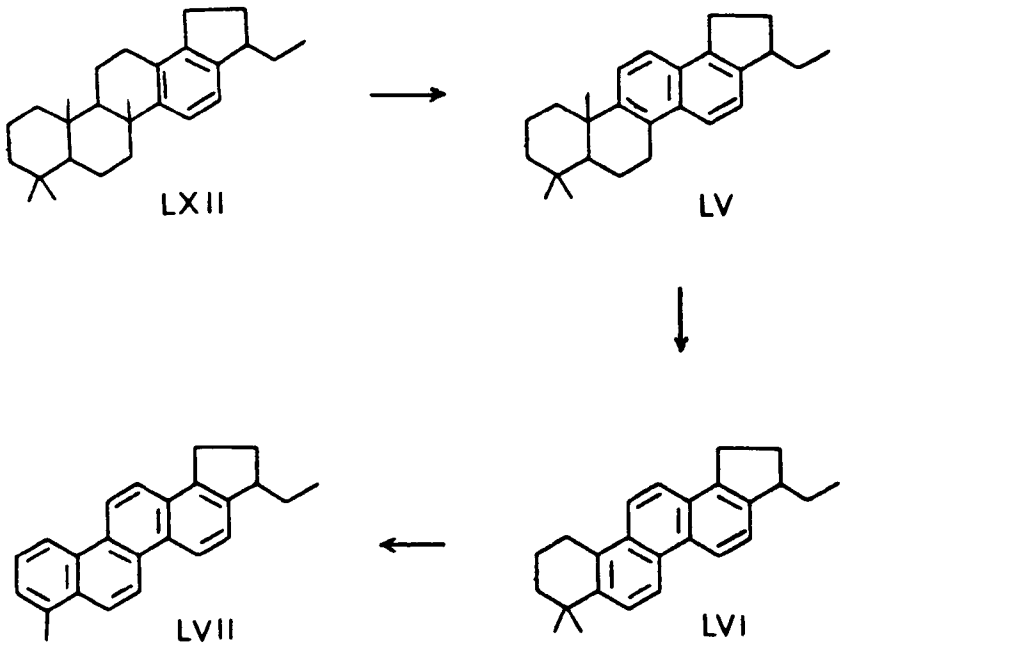
6.3.ii Lipid diagenesis

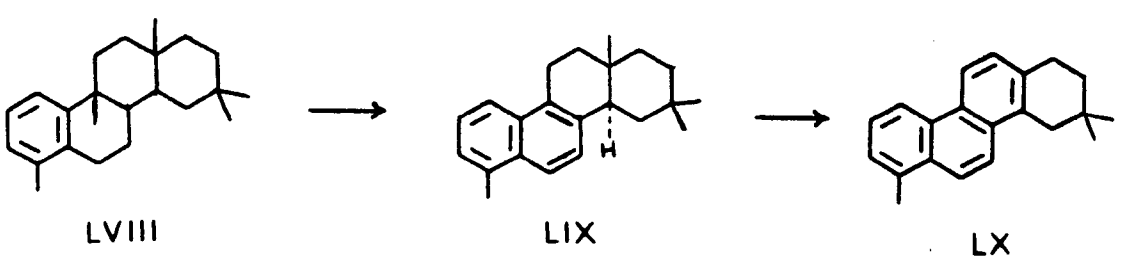
The high organic carbon content of Messel oil shale demonstrates that it was deposited under highly productive conditions, which would have favoured lipid preservation. The mild burial temperature history has also resulted in relatively little degradation of organic matter, resulting in survival of lipids unaltered from the original biolipids. Lipid indicators of the maturity of the Messel oil shale include the presence of unsaturated compounds, the abundance of functionalised molecules, the abundance of hopanes having the unstable 17β(H),21β(H)-stereochemistry and the low levels of hopanes having a 17α(H),21β(H)- or 17β(H),21α(H)-stereochemistry. The low level of maturity of the Messel oil shale contrasts with that of the well documented Green River Formation oil shale (e.g. Bradley, 1970), of similar (Eocene) age.

The very low abundances of desmethylsterols and non-hopanoid

triterpenoids indicates that the contribution from higher plants was minimal and suggests that diagenesis may have modified the distributions of the homologous series of n-alkanes and n-alkanols, with shorter chain homologues having been preferentially degraded, thus enhancing the apparent higher plant-derived input of $>C_{21}$ lipids. The n-alkanes may have been formed by decarboxylation of the fatty acid methyl esters in view of their similarity of distribution (cf. Figs. 6.2/2 and 6.2/4). No similarity of distribution was observed between the alkan-2-ones and the n-alkanes.

Partially to fully aromatised hopanoids in sediments have been postulated to arise by a diagenetic sequence of successive aromatisation from ring D through rings C, B and A (Greiner et al., 1976,1977; Spyckerelle et al., 1977a,b). Thus in Messel shale the tetraaromatic hopanoid (LVII) would have been formed by further aromatisation of the triaromatic hopanoid (LVI), and, at the diagenetic stage of the Messel shale used in the present study, mono- and diaromatic hopanoids are negligible. 3,3,7-Trimethyl-1,2,3,4-tetrahydrochrysene (LX) was probably formed soon after deposition in the palaeoenvironment of Messel by loss of ring A of a 3-oxygenated triterpenoid, such as olean-12-en-3-one or taraxer-14-en-3-one, initiated either by photochemical or by photomimetic oxidation, and followed by aromatisation through rings B to D (Corbet et al., 1980).


LXII
LV
LVII
LVI


H
LVIII
LIX
LX

In sediments 17β(H),21β(H)-hopanes can undergo epimerisation to give the thermodynamically more stable 17β(H),21α(H)-hopanes and, with further burial temperature increases, the 17α(H),21β(H)-hopanes are formed and predominate. Extended hopanes may also exist as 22R or 22S diastereomers. Thus the presence of 22R-17α(H),21β(H)-homohopane in Messel shale may reflect diagenetic transformation of 17β(H),21β(H)-homohopane, however, the presence of only one 17α(H),21β(H)-hopane, which occurs as a single diastereomer, the absence of any 17β(H),21α(H)-hopanes and the presence of abundant hopenes, suggest that it might have arisen through direct input, especially as 17α(H),21β(H)-homohopane, present only as the 22R form, has been identified in a peat (Taylor et al., 1981).

Redox reactions performed in the laboratory on the sedimentary alcohols and ketones demonstrated that the distributions of 4α-methylsterols and 4α-methylsteroidal ketones were essentially parallel (Fig. 6.3/1), demonstrating the common biosynthetic origin of these two compound classes. 4α-Methylsteroidal ketones have been identified in dinoflagellates (Withers et al., 1978; Robinson et al., 1984a; Chapter 3, this thesis); thus, dinoflagellates could have provided a direct biological origin for the 4α-methylsteroidal ketones present in Messel shale, or, alternatively, they may have been produced by a non-selective microbial oxidation of the sedimentary 4α-methylsterols.

Laboratory conversion of the sedimentary 4α-methylsterols to hydrocarbons produced a series (C_{28} to C_{30}) of 4α-methylsteranes with a similar distribution to that reported in Messel shale by

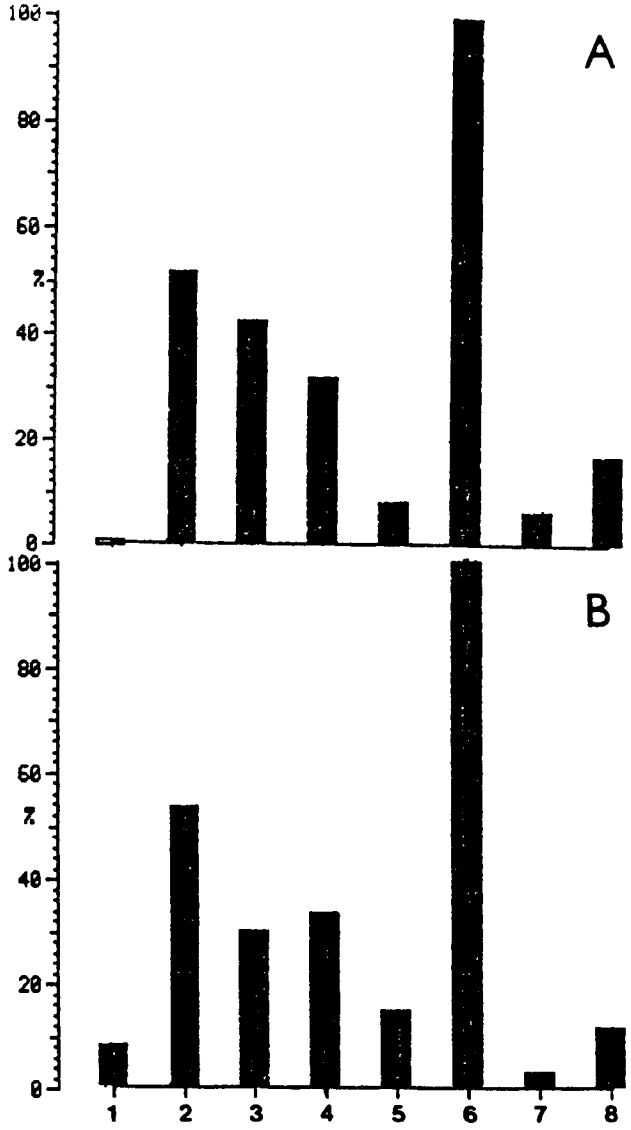


Fig. 6.3/1. Distribution of (A) 4α-methylstanols and (B) 4α-methylsteroidal ketones in Messel oil shale.
Identities:-

1 = 4α-methyl-5β(H)-cholestan-x
2 = 4α-methyl-5α(H)-cholestan-x
3 = 4α,24-dimethyl-5α(H)-cholestan-x
4 = 4α,23,24-trimethyl-5α(H)-cholest-22-en-x
5 = 4α-methyl,24-ethyl-5α(H)-cholest-22-en-x
6 = 4α,23,24-trimethyl-5α(H)-cholestan-x*
7 = 4α,23-dimethyl,24-ethyl-5α(H)-cholest-28-en-x
8 = 4α,23-dimethyl,24-ethyl-5α(H)-cholest-28-en-x

x = 3β-ol or 3-one

* includes an unkown contribution from 4α-methy,24-ethyl-5α(H)-cholestan-x.

Kimble et al. (1974a). Additional compounds produced were 4α-methylsterenes corresponding to the 4α-methylsterols with side-chain double bonds. These results provide additional evidence supporting the hypothesis that sedimentary steranes arise from sterol diagenesis (Mackenzie et al., 1982 and references therein). The C_{30} 4α-methylsteranes, I′ and K′ in Fig. 6.2/4, had similar spectra, but were tentatively assigned as 4α,23,24-trimethyl-5α(H)-cholestane and 4α-methyl,24-ethyl-5α(H)-cholestane, respectively, from their mass spectra and by analogy with the elution order of the parent 4α-methylstanols. Coupled with an elution time difference of only 18 seconds under the the GC conditions used, the similarity of mass spectra makes the identification by Kimble et al. (1974a) of the sedimentary C_{30} 4α-methylsterane as having a 24-ethyl side-chain open to question, especially as the GC columns available to those workers were much less efficient than they are today. Further work is required to confirm the structure of the C_{30} 4α-methylsterane in Messel oil shale as either having a 24-ethyl side chain or a 23,24-dimethyl side chain (or the presence of both compounds in the shale); such work is currently in progress in this laboratory. The recognition of a major dinoflagellate input to Messel oil shale suggests that the presence of a 4α-methylsterane having a 23,24-dimethyl side chain is very likely, based on known sterol contents of such organisms and the hypothesis that steranes arise via transformation of sterols.

6.3.iii Comparison of results with other published Messel oil shale results

In common with other published work, this investigation has shown Messel oil shale to be an organic rich sediment with a markedly lower maturity than the well-documented Green River Formation oil shale, U.S.A., of the same age (e.g. Kimble et al., 1974a). Functionalised compounds were abundant, with sterols and steroidal ketones being dominated by 4α-methylsteroids, consistent with previous reports (Mattern et al., 1970; Habermehl and Hundrieser, 1983a).

The absence of the mono- and diaromatic hopanoids (LIV and LV) and the mono- and diaromatic tetracyclic hydrocarbons (LVIII and LIX) reported in Messel shale by previous workers (Greiner et al., 1976, 1977; Spyckerelle et al., 1977a,b), may be due to the sample of Messel shale used in the present study having a greater maturity. In contrast, the absence of 4α-methylsteranes and a lower abundance of 17α(H),21β(H)-hopanes compared with the sample studied by Kimble et al. (1974a), appears to indicate that our sample of Messel shale is of lower maturity than that studied by Kimble et al.. Unpublished results of this laboratory and others (Dr. J. De Leeuw, personal communication), show that Messel oil shale is very inhomogeneous in its lipid content, which may be due to maturity differences or, since deposition is believed to have taken place as a series of shallow, swampy lakes, to

differences in input and microbial populations in various parts of the basin.

Albrecht (1969) reported isoprenoid alkanes in the relative order of abundance $C_{16}>C_{20}>C_{18}>C_{19}>C_{15}$. The same suite of isoprenoid alkanes was recognised in the present study, although with a different order of relative abundance $C_{16}>C_{15}>C_{20}>C_{19}>C_{18}$. Also the relative abundance of $\underline{n}$-C_{23} alkane was found to be greater than that reported by Albrecht (1969). The identification of a series of ethyl esters by Habermehl and Hundrieser (1983) was not repeated, nor was the identification of C_{22} and C_{25} 4α-methylsterols and 4α-methylsteroidal ketones by the same authors.

Previously the C_{30} 4α-methylsterol and 4α-methylsteroidal ketone present in Messel shale have been reported to have a 24-ethyl side-chain (Mattern et al., 1970; Habermehl and Hundrieser, 1983a). The present study has shown the major steroid in a particular sample of Messel oil shale to be a saturated C_{30} 4α-methylsteroid, with both the 23,24-dimethyl and 24-ethyl side-chain compounds occurring, the former probably dominating. Both side-chain variants (as sterol or ketone) have similar mass spectra and GC elution times, indeed on the packed columns used by Mattern et al. (1970) the two isomers would probably coelute. In the absence of published detailed experimental conditions used by Habermehl and Hundrieser (1983a) it is not possible to say whether the compounds they observed did possess a 24-ethyl containing side-chain, or were misassigned. 4α,23R,24R-Trimethylsteroids are more consistent with the postulated dinoflagellate input to Messel, in view of the known

occurrence of dinoflagellate steroids (Withers, 1983; Chapter3, this thesis).

No C_{31} steroidal ketones have been reported previously, although 4α-methylgorgostanol has been claimed to occur in Messel shale (Habermehl and Hundrieser, 1983a). 4α-Methylgorgostanol was not identified in the present study, however, a novel C_{31} 4α-methylsterol was recognised, existing as two isomers, tentatively identified as 24R- and 24S- 4α,23-dimethyl, 24-ethyl-5α(H)-cholest-28-en-3β-ol; the corresponding ketones were also detected. No further comment can be made upon the claimed identification of 4α-methylgorgostanol in Messel shale (Habermehl and Hundrieser, 1983a) in the absence of published detailed experimental conditions.

6.4 CONCLUSIONS

The results of this study confirm much of the previously reported (e.g. Mattern et al., 1970; Kimble et al., 1974a: Habermehl and Hundrieser, 1983a) data on the Messel oil shale and provide new information on lipid composition and origins. Certain differences with other studies may be explained by the inhomogeneity of the shale and/or by incomplete identifications made by earlier workers. The main conclusions which may be drawn from this study are as follows:-

1) The Messel oil shale is of a low maturity reflecting its burial temperature history of no more than 40 °C (Kimble et al.,

1974a).

2) 4α-Methylstanols are present in abundance and are postulated to originate from a dinoflagellate input in the palaeoenvironment of deposition 50 million years ago.

3) 4α-Methylsteroidal ketones are present, which correspond to the 4α-methylstanols; these ketones may originate from a direct input or from oxidation of the 4α-methylstanols.

4) Hopanoids are abundant, present as alkanes, alkenes, aromatic hydrocarbons, alcohols and ketones, and reflect a significant bacterial contribution to the sediment.

5) Mid-chain ketones were recognised; they may represent a higher plant input. The overall higher plant input is believed to have been relatively low. The previously oldest sediment in which mid-chain ketones have been reported was of the order of 10000 years old (Cranwell, 1984).

6) An extended series of alkan-diols and hydroxyalkanones was isolated; they are the oldest yet reported, their origin is unknown.

7) A novel C_{31} 4α-methylsterol, present as two stereoisomers, was tentatively identified as 4α,23-dimethyl,24-ethyl-5α(H)-cholest-28-en-3β-ol by mass spectral interpretation of its TMS derivative, together with the corresponding ketones. These compounds may share a common origin with the other 4α-methylsteroids from dinoflagellates.

8) Laboratory conversion of 4α-methylstanols to 4α-methylsteroidal hydrocarbons, resulted in a very similar distribution of 4α-methylsteranes to that reported for the Messel oil shale (Kimble et al., 1974a; Wardroper et al., 1977), thus

providing further support for the hypothesis that steranes in sediments arise from diagenetic alteration of sterols.

CHAPTER SEVEN

OVERALL CONCLUSIONS

7.1 THIS WORK

7.1.i General

The origin and fate of lipids in lacustrine environments have been investigated. Microorganisms have been shown to play a very important role in a number of lacustrine environments as both source organisms of sedimentary lipids and as the major agents producing the early stages of diagenesis. The enclosed nature and generally higher productivity of lakes compared with open ocean areas, have enabled the lipids of natural populations of microorganisms collected from the waters of Priest Pot and Lake Kinneret to be compared directly with the sedimentary lipid distributions (see sections 4.3 and 5.3). Such comparisons have resulted in the recognition of classes of compounds, present in sediments, which act as biological marker compounds for input from specific groups of organisms. Similarly, certain compounds, isolated from the natural populations of organisms, were not preserved in the underlying bottom sediments, due to degradation and transformation within the water column or soon after incorporation into the sediments. The studies on natural populations of organisms have been complemented by the analysis of two cultured freshwater dinoflagellate species (see Chapter 3). Many of the compounds identified are novel or have been detected in the lacustrine environment for the first time.

7.1.ii Organisms

Analysis of the extractable lipids of Peridinium lomnickii, P. cinctum, Ceratium hirundinella and Woloszynskia coronata has demonstrated that freshwater dinoflagellates are capable of biosynthesising a wide range of 4α-methylsterols and desmethylsterols, in common with marine species (Withers, 1983 and references therein). A dinoflagellate origin for sedimentary 4α-methylsterols was proposed based on their similarity of distribution in a natural population of P. lomnickii, collected from the waters of Priest Pot, and in the underlying bottom sediments (Fig. 4.3/1). Support for this proposal was provided by the occurrence of relatively abundant levels 4α-methylsterols in the bottom sediments of Lake Kinneret (Fig. 5.3/1), consisting of a similar suite to that isolated from P. cinctum, the dominant member of the lake's phytoplankton. Furthermore, 4α-methylsterols were observed to be absent in the sediments of Coniston Water, a lake which does not support a significant dinoflagellate population. The presence of 5α(H)-cholestan-3β-ol as a major sterol of P. lomnickii demonstrates that dinoflagellates may be the long sought after direct biological source of sedimentary 5α(H)-stanols. In the marine environment, sterols with a cyclopropyl containing side-chain have long been regarded as resulting from dinoflagellate biosynthesis (Alam et al., 1979a; Withers et al., 1979a; Kokke et al., 1981b; Withers, 1983). The isolation of gorgosterol (VI m) and gorgostanol (III m) from C. hirundinella provides the first report of such

compounds occurring in a freshwater alga and proves that sterols with the cyclopropyl group are biosynthesised by lacustrine organisms, namely dinoflagellates. The novel sterol, 4α-methylgorgosterol (XIII m), was isolated from the sediments of Lake Kinneret and probably originates from dinoflagellates. Peridinosterol (XII l), occuring in P. cinctum, has not previously been identified in a freshwater organism; its detection in the sediments of Lake Kinneret shows that it is a potential biological marker compound for a restricted group of dinoflagellate species.

Previously, the only 4α-methylsteroidal ketone known to occur in algae was dinosterone (XVI j), which had been isolated from the marine dinoflagellate Crypthecodinium cohnii (Withers et al., 1978) . Sedimentary steroidal ketones were thought to arise from two major sources: formation by microbial degradation of sterols, as demonstrated by radiolabelling studies (Edmunds et al., 1980); and contribution as direct biolipid inputs from planktonic or other source organisms, despite the sparsity of such reported occurrences (Gagosian and Smith, 1979). 4α-methylsteroidal ketones, however, are major components of the free ketone fractions isolated from P. lomnickii and W. coronata. The similarity of the distributions of 4α-methylsteroidal ketones in P. lomnickii and Priest Pot sediments (Fig. 4.3/3), demonstrates that dinoflagellates can be a major source of such compounds in sediments. In Lake Kinneret, the dominant member of the phytoplankton, P. cinctum, does not produce 4α-methylsteroidal ketones and the sedimentary 4α-methylsteroidal ketone:4α-methylsterol ratio is much smaller than in Priest Pot. The range and distribution of sterols and steroidal ketones

present in the four species of freshwater dinoflagellates studied, provides new information on the biosynthesis of such compounds, in particular the order of bioalkylation and ring saturation and the possibility of a number of complex interconnected pathways leading to steroid formation.

Evidence is accumulating that steryl esters may occur widely in dinoflagellates (Teshima et al., 1980; Kokke et al., 1981b; Volkman et al., 1984). Until recently (Wakeham and Frew, 1982) analysis of steryl esters involved saponification and identification of the resulting sterols and fatty acids, which precluded recognition of individual constituents. Each of the four freshwater dinoflagellates studied herein contained steryl esters, which have been analysed directly as intact molecules, making use of on column injection for capillary GC, bonded-phase GC columns of high thermal stability and the development of improved interfaces between the GC and mass spectrometer. Similarly, triacylglycerols, the primary metabolic energy reserve of most phytoplankton (Sargent, 1976), have been isolated from freshwater dinoflagellates and analysed without hydrolysis.

Although steroids were major constituents of the dinoflagellate species studied, other compound classes, including methyl and ethyl esters, were significant components of P. cinctum, C. hirundinella and W. coronata. Unlike steroids, however, many of these other compounds were not preserved in the sediment, e.g. heneicosahexaene was the dominant hydrocarbon of P. cinctum, C. hirundinella and W. coronata, but was not detected in sediments. Comparison of the lipids of natural populations of rotifers, ciliated protozoa and Clathrochloris hypolimnica bacteria, collected from the waters of Priest Pot, with the

lipids of the underlying bottom sediments, revealed that these aquatic microorganisms contributed only a small proportion, mainly heptadecane, of the sedimentary hydrocarbons, with the majority of the sedimentary hydrocarbons originating from higher plants (Section 4.3.iii). $\Delta^{5,7}$ and Δ^{7} sterols were isolated from the rotifers and ciliated protozoa and were assigned to an origin from feeding of these organisms on Chlorophyceae, but only Δ^{7} sterols were preserved in the sediments of Priest Pot. Significant populations of Chlorophyceae occur in Lake Kinneret, and, as in Priest Pot, the sediments were found to contain Δ^{7} sterols, but not $\Delta^{5,7}$ sterols. The $\Delta^{5,7}$ sterols appear to be removed by zooplankton feeding processes with the Δ^{7} sterols being largely unaffected by passage through the gut of zooplankton, a phenomenon which has been demonstrated in marine zooplankton by laboratory feeding studies (Prahl et al., 1984; Neal, 1984).

7.1.iii Lake sediments

The sedimentary lipids of Coniston Water, Priest Pot and Lake Kinneret were found to reflect their trophic status, bathymetry and the vegetation of their catchment area. Thus Coniston Water, which is the least productive of the three lakes, has the highest proportion of terrestrially derived sedimentary lipids. Lipids of higher plant origin were relatively more abundant in Priest Pot sediments than in the sediments of Lake Kinneret, even though Priest Pot has a higher productivity, presumably because of the sparsity of vegetation on the catchment area of Lake Kinneret and

because the sediments were collected from near the centre of the lake, removed from inflows of terrestrial material. The distribution of lipids by compound type is shown in Fig. 7.1/1.

The early stages of lipid diagenesis in the lacustrine environment were investigated by comparing the lipid distributions of source organisms with those of the surface sediments of Coniston Water, Priest Pot and Lake Kinneret, and by determining the changes in the sedimentary lipids with increasing depth of burial over a short range, spanning a period of up to 80 years. In each of the sediments studied the early stages of lipid diagenesis were characterised by intense microbial activity producing rapid changes compared with the slow physico-chemical changes, brought about by the agencies of increasing temperature and pressure, which take place at greater burial depths. Shorter chain homologues and unsaturated compounds were observed to be preferentially removed, consistent with previous findings (Quirk, 1978; Kawamura et al., 1980; Cranwell, 1981a; Cardoso et al., 1983). Previous workers have noted that preservation of organic matter is greater in lakes with anoxic water columns and high sedimentation rates (Didyk et al., 1978; Cranwell, 1982), because in such lakes predation and bacterial degradation are decreased by the anoxic conditions and as the sedimentation rate results in rapid removal from the sediment/water interface, where bacterial concentrations are greatest. Results obtained from the lakes studied herein were consistent with the above observations, viz. preservation of labile compounds is better in the sediments of Priest Pot and Lake Kinneret, the productivity and bathymetry of which produce anoxic sediments and seasonally anoxic hypolimnia, than in the sediments of the oligo-mesotrophic Coniston Water.

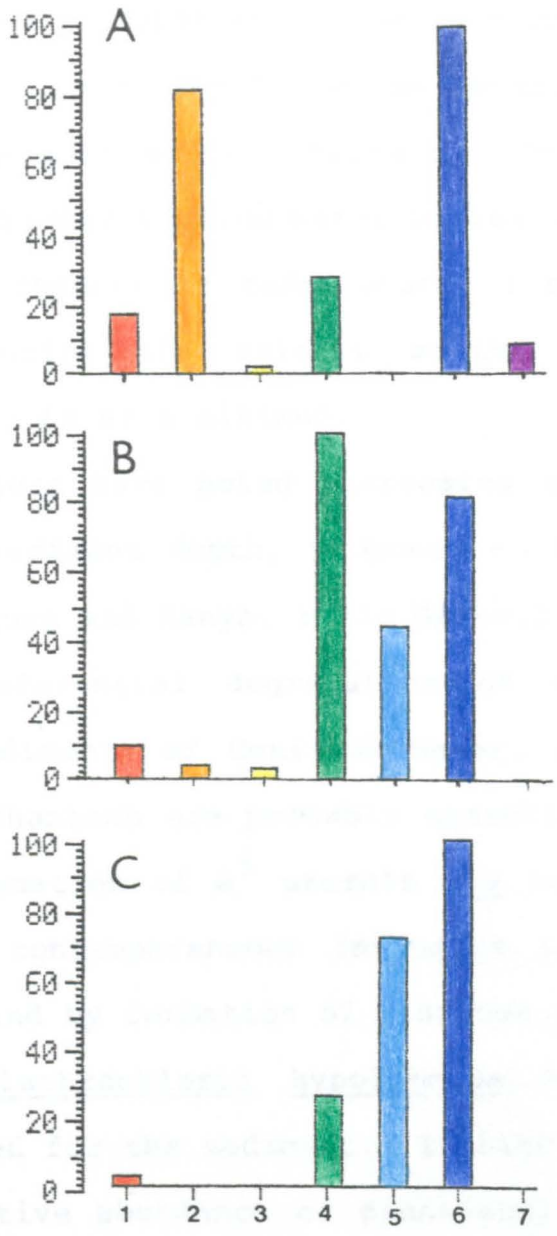


Fig. 7.1/1. Relative abundance of free lipids by compound class in three lake sediments: (a) Coniston Water (0-3 cm)
(b) Priest Pot (0-6 cm)
(c) Lake Kinneret (surface sediments)

Key:- 1 = Hydrocarbons
2 = Fatty acids
3 = Ketones
4 = Alkanols
5 = 4α-methylsterols
6 = desmethylsterols
7 = Hydroxy acids

Overturn does normally occur during the winter months in both Priest Pot and Lake Kinneret resulting in a completely oxygenated water column, but the sediment remains anoxic except for a thin layer at the sediment/water interface. Priest Pot and Lake Kinneret are relatively shallow water bodies and their hypolimnia exhibit seasonal changes in temperature, turnover of the water column occurs during the coldest months of the year when microbial activity is at a minimum.

Previous workers have noted increasing stanol:stenol ratios with increasing sediment depth, proposed to be due to microbial hydrogenation (Ogura and Hanya, 1973; Gaskell and Eglinton, 1975, 1976) or to preferential degradation of stenols (Nishimura, 1977). In the sediments of Coniston Water, Priest Pot and Lake Kinneret both mechanisms are probably operating. In Priest Pot, bacterial hydrogenation of Δ^5 sterols *via* ketone intermediates, is supported by contemporaneous increases in 5β(H)-stanols and 5α(H):Δ^5 ratios and by formation of stanones and 3α-OH stanols in the bacterial *Clathrochloris hypolimnica* layer, with further increases observed for the sediment. In Lake Kinneret, the large increase in relative abundance of dinostanol (XII j) between *P. cinctum* and the surface sediment, followed by a small increase in abundance in the 15 cm deep sediment, provides good evidence that this stanol may be formed by microbial hydrogenation of dinosterol (XII i) in the sediments of Lake Kinneret. In both Priest Pot and Lake Kinneret sediments 4α-methylsterols were proposed to resist degradation better than desmethylsterols, based on the observed increase in relative abundance of 4α-methylsterols in the deeper sediment section of each lake. A large increase in relative abundance of 4α-methylsterols was also

observed between P. cinctum and Lake Kinneret bottom sediments. 4α-Methylsterols have been proposed to be more resistant to bacterial degradation than desmethylsterols in the marine environment (Gagosian et al., 1980); the results obtained from this study suggest that 4α-methylsterols are more resistant to degradation from lacustrine bacteria, but that enhancement of 4α-methylsterols by zooplankton feeding processes may also be a contributing factor.

Although extraction procedures involving saponification, transesterification or demineralisation were formerly used in the isolation of sedimentary lipids, it is now recognised that maximum information can only be obtained if the extraction procedure allows the isolation of free, esterified and bound lipids separately (Farrington and Quinn, 1971, 1973; Nishimura, 1977; Cranwell, 1978). Bound lipids isolated from Coniston Water and Priest Pot sediments resembled each other more closely than the corresponding free lipids. A greater bacterial contribution to the bound lipids than the free was recognised, e.g. relatively greater abundances of iso- and anteiso-branched fatty acids and alkanols (cf. Parker, 1968; Cranwell, 1980); abundant 3-hydroxy acids of microbial origin (see Cranwell, 1981b; Cardoso and Eglinton, 1983; Klok, 1984). Such findings are consistent with results obtained from other lake sediments (Brooks et al., 1976; Cranwell, 1978, 1979, 1981a; Cardoso et al., 1983). As in other lake sediments (Cranwell, 1981a), bound lipids were found to be stabilised relative to free lipids, e.g. smaller depth related changes in stenol:stanol ratios were observed for bound compared with free lipids; unsaturated bound acids and alcohols were more persistent with increasing sediment depth, and hence

burial time; shorter chain lipids were preserved in the bound lipid fractions, whereas they were preferrentially degraded in the free lipid fractions. In Priest Pot and Coniston Water sediments, in situ formation of bound lipids by conversion of free lipids apparently takes place.

7.1.iv Messel oil shale: an ancient lacustrine sediment

The results obtained from this study of the lipids of freshwater organisms and contemporary lake sediments were applied to the 50 x 10^6 years old lacustrine Messel oil shale. The free lipids of this ancient lacustrine sediment showed many similarities to the lipids of modern lake sediments, containing similar suites of alkanes, alkanoic acids, ketones, alkanols, steroids and triterpenoids, thus supporting the hypothesis that modern lakes may be analogous environments of deposition to those which resulted in the formation of certain oil shales (Bradley, 1966). A major dinoflagellate population in the palaeoenvironment of deposition of Messel oil shale was inferred from comparison of the distributions of 4α-methylsterols and 4α-methylsteroidal ketones isolated from the shale, with those observed in present day dinoflagellates. Improvements in GC-MS instrumentation enabled the structures of the C_{30} 4α-methylsteroids present in Messel oil shale to be assigned to a 4α,23,24-trimethylsteroid skeleton, as well as the previously proposed (Mattern et al., 1970; Kimble et al., 1974a; Habermehl and Hundrieser, 1983a) 4α-methyl,24-ethylsteroid skeleton. A large bacterial contribution to Messel oil shale was noted, but, unlike to

previous reports, a relatively small higher plant input.

7.2 FUTURE WORK

Comparison of the lipids isolated from natural populations of organisms (P. lomnickii, P. cinctum, rotifers, ciliated protozoa, C. hypolimnica and Elodea nultalli) with the lipids of underlying bottom sediments (Priest Pot, Lake Kinneret and Coniston Water) has proved a useful and informative approach to the characterisation of the sources and fate of organic matter in sediments. Extension of this type of study to further organisms and sediments is desirable; particularly suitable organisms might be members of the Chlorophyceae. Where it is not feasible to collect natural populations of organisms, cultures provide a useful alternative. More information is required on changes in lipid content of organisms at different growth stages and under varying physiological conditions, e.g. dinoflagellate cysts. The present study has inferred modification of algal lipids by zooplankton feeding processes and/or bacterial attack during sedimentation, laboratory feeding studies could be performed using freshwater organisms, thus investigating the relative biodegradability of $\Delta^{5,7}$ sterols and Δ^{7} sterols and of 4α-methylsterols and desmethylsterols. Much more information, both biological and chemical, is desirable on the sedimentary bacteria, which can exert a very large influence on the lipid composition of sediments.

Complete characterisation of some of the lipids detected in

this work (e.g. the C_{28} stanols detected in Lake Kinneret sediments and the novel C_{31} steroids tentatively identified in Messel oil shale) requires synthesis of standard compounds or, alternatively, preparative scale HPLC of natural lipid fractions to isolate single compounds in sufficient quantity for structural elucidation by techniques such as nmr. Conversion of naturally occurring sterols to steroidal hydrocarbons, as described in chapter 6, could be applied to other sedimentary sterol fractions, e.g. to produce cyclopropyl containing steranes from sterols related to gorgosterol to investigate their GC and mass spectrometric properties.

CHAPTER EIGHT

EXPERIMENTAL

8.1 GENERAL

General purpose solvents were distilled before use and double distilled water was obtained from a two-stage all-glass still. Glassware was cleaned by soaking in Decon 90 (BDH) overnight, rinsing with tap water followed by double distilled water, dried and rinsed with an appropriate solvent before use. Dry solvents used in chemical reactions, such as toluene, pyridine, tetrahydrofuran, diethyl ether, etc., were heated under reflux with either sodium or $LiAlH_4$ and then distilled, discarding the first and last 10% of the distillate. All solvents, once dry, were stored over 5Å molecular sieves. Molecular sieves are a possible source of contamination, but this was not found to be a problem using solvents stored in this way for syntheses; where lower amounts of material are used, pre-extraction of the molecular sieves with a suitable solvent would be advisable. HCl was stored over dichloromethane, KOH was fused prior to use, $MgSO_4$ was extracted with dichloromethane and oven-dried. Contaminants were checked for by blank extractions; no significant peaks were observed in GC of the blank extraction fractions except for samples which had been treated with BSTFA, but such peaks did not affect recognition of natural compounds in GC-MS analysis of lipid fractions treated with BSTFA. Plasticisers, notably of the phthalate type, characterised by a strong m/z 149, were observed at low levels in GC-MS analysis of some samples; when encountered such compounds were disregarded as contaminants.

8.2 COLLECTION OF SAMPLES

Sediment cores were obtained with a 1m Mackereth corer (Mackereth, 1969), stoppered with rubber bungs and transported to the laboratory where they were extruded pneumatically and sectioned. Following centrifugation to remove excess water, sediment samples were stored frozen (-20 °C). Natural populations of organisms were collected from the waters of Priest Pot using plastic tubing connected to a peristaltic pump; concentrated samples for further work up were obtained by centrifugation or filtration. Peridinium cinctum and Lake Kinneret sediment samples were collected by Dr. M.Gophen. No detail is available regarding collection of the Messel sample used in the present study.

8.3 EXTRACTION

Wet sediment samples were generally extracted with MeOH-$CHCl_3$ (ultrasonication) followed by exhaustive extraction with $CHCl_3$ using a Soxhlet apparatus. Bound lipids were released by heating the extracted sediment with 6N HCl under reflux. The acid was decanted and extracted by shaking with dichloromethane; the hydrolysed sediment was extracted with MeOH-$CHCl_3$ (ultrasonication) and combined with the extract of the acid.

Rotifers and ciliated protozoa were extracted with methanolic KOH (ca. 5%, 5 hours). Clathrochloris hypolimnica, Elodea nultallii and Ceratium hirundinella were extracted with MeOH-$CHCl_3$ (ultrasonication). Freeze-dried samples (Kinneret

sediment and Peridinium cinctum) were extracted with dichloromethane (ultrasonication).

Separation of acidic and neutral components was achieved by partition between aqueous KOH and dichloromethane; acidic components were recovered by acidifying the aqueous layer to pH 1 and extraction with dichloromethane.

8.4 ANALYTICAL TECHNIQUES

8.4.i Liquid chromatography

Columns were prepared with a slurry of alumina (BDH, Brockmann activity II) in hexane. The alumina was pre-eluted with hexane during preparation of the column. The sample was applied to the top of the column as a dry dispersion on alumina and was eluted with successive solvent(s) of increasing polarity.

8.4.ii TLC

Preparative TLC plates (20 x 20 cm; 0.5 mm thickness) were prepared by spreading glass plates, six at a time, with a slurry of silica gel G (Merk) in deionised water and allowing to dry. Plates were pre-eluted with ethyl acetate and reactivated (120°C, 30 minutes) prior to use. Standards were applied to the right hand side of the plate separated from the sample, and were visualised with Rhodamine G. Recovery of lipids was accomplished by elution with a solvent of appropriate polarity through a short

alumina column.

8.4.iii Gas chromatography

Most analyses were performed on a Carlo Erba 4160 chromatograph using an on-column injector. Open-tubular Flexsil columns (25m x 0.3mm or 50m x 0.3mm) wall-coated with an apolar liquid phase (either OV1 or CPSil 5) were used. A typical run was temperature programmed from 80-300 °C at 4 min^{-1}. Hydrogen was employed as carrier gas, the flow rate being adjusted according to the column used; compounds were detected with a flame ionisation detector heated to the maximum temperature of the column. Samples were applied dissolved in dichloromethane.

8.4.iv Gas chromatography-mass spectrometry

The majority of the analyses were performed using a Finnigan 4000 mass spectrometer coupled to a Finnigan 9610 gas chromatograph with on-line Incos 2000 data system and a Printronix printer (Brassell et al., 1980b); later analyses were performed with a Carlo Erba Mega gas chromatograph, fitted with an on-column injector, replacing the Finnigan 9610. Typical operating conditions were:

scan range: m/z 50-550
scan time: 1s
filament current: 0.35mA
electron voltage: 35-40eV
accelerating voltage: 1-2kV

source temperature: 250°C

GC conditions were similar to those described in section 8.4.iii, but with helium replacing hydrogen as carrier gas.

8.4.v HPLC

HPLC was performed with a Spectra Physics 8700 solvent programmer. Eluting peaks were observed with a variable wavelength UV/VIS spectrophotometer (LDC, Spectromonitor II). Quantitation was achieved *via* an on-line VG Datasystem 5000. A stainless steel column (25cm x 4mm i.d.) packed with Hypersil (3um particle size) was used for both analytical and preparative HPLC.

8.4.vi Optical polarimetry

Optical rotations were measured on a Model 241 MC Polarimeter (Perkin-Elmer) in a solution of dichloromethane.

8.4.vii Urea adduction

A saturated solution of urea in methanol (0.5ml) was added dropwise to a solution of the sample in hexane (4ml) and acetone (2ml). After centrifugation (1500 rpm, 15 min) the solvent was removed under a stream of nitrogen. The non-adduct was obtained by washing the resulting crystals with hexane (3 x 5ml) and filtering through a small plug of pre-extracted cotton wool in a pasteur pipette. The adduct was recovered by dissolving urea in

water (5ml) and extracting with hexane (3 x 5ml). Where necessary the adduction procedure was repeated.

8.5 CHEMICAL TECHNIQUES

8.5.i Silylation

Hydroxy groups were converted to trimethylsilyl ethers (TMS) prior to GC analysis. BSTFA [Bis(trimethylsilyl)-trifluoroacetamide] (Pierce; ca. 25 ul) was added to the dry sample in a vial. The sealed vial was placed in a warm place (top of gas chromatograph, ca. 1 hour) and excess BSTFA removed under a stream of nitrogen. If a hindered hydroxyl group was present, derivatisation took place in pyridine (ca. 1ml) at 60°C overnight.

8.5.ii Methylation

Carboxylic acids, typically of the order of 1 mg, were converted to methyl esters by heating under reflux (15 min) with BF_3/MeOH (BDH) (ca. 15 ml). The resulting methyl esters were evaporated to dryness under reduced pressure, dissolved in water, extracted with dichloromethane and purified by TLC.

REFERENCES

8.5.iii Hydrogenation

The method of Zielinski *et al.* (1983) was followed. The sample (*ca.* 2mg) was dissolved in hexane (4ml) and glacial acetic acid (1ml). Platinum dioxide (*ca.* 2mg) was added and the mixture stirred (4 hours) under a flow of hydrogen (1 atm.) at room temperature. Double distilled water was added to the reaction mixture, followed by extraction with hexane-diethyl ether (1:1, 4 x 10ml). The extract was dried with pre-extracted anhydrous $MgSO_4$, filtered and evaporated to dryness under reduced pressure.

8.6 SYNTHESES

8.6.i Synthesis of 2′-hexadecyl-2S-acetylmandelate

A modification of the method of Hitchcock and Rose (1971) was employed. S-Mandelic acid (Aldrich, Gold label) and excess acetyl chloride were mixed and warmed gently until a clear solution resulted. Excess acetyl chloride was removed under reduced pressure and the residue dissolved in toluene. Oxalyl chloride added, mixture left at room temperature (1 hour) and heated under reflux (2 hours). Evaporation of the solvent afforded an orange-yellow oil (S-acetylmandelyl chloride).

Racemic hexadecan-2-ol (184mg), dissolved in dry pyridine (5ml), was treated with excess of the acid chloride. The mixture was placed in a dessicator (room temperature, 20 hours), then diluted to 30ml with diethyl ether, washed with aq. ammonium

chloride (2 x 20ml), aq. calcium carbonate (2 x 20ml) and water (2 x 20ml) and dried over anhydrous magnesium sulphate. Purification by liquid chromatography (alumina) followed by TLC (silica gel G) afforded 2′-hexadecyl-2S-acetylmandelate as a yellow oil in 25% yield, the identity of which was checked by mass spectrometry.

8.6.ii Synthesis of R-trans-chrysanthemate esters

The esters were prepared according to the method of Brooks et al. (1973). R-trans-chrysanthemic acid was treated with freshly distilled thionyl chloride in a sealed vial (60 °C, 1 hour). Excess thionyl chloride removed under a stream of nitrogen. The acid chloride was treated with alcohol dissolved in dry toluene in a sealed vial (40 °C, 2 hours) and the product, produced in quantitative yield, purified by TLC [silica gel G; 0.5mm; hexane-diethyl ether (4:1)].

8.6.iii Synthesis of ethyl n-hexadecanoate

Hexadecanoic acid (200mg) was heated under reflux (2 hours) with ethanol (20 ml) containing a few drops of conc. H_2SO_4. The product was purified by recrystallisation from aq. ethanol to give a white crystalline solid (65%) producing one peak on GC and with a mass spectrum characteristic of a C_{18} ethyl ester.

8.7 COMPOUND CLASS INTERCONVERSIONS

8.7.i Oxidation of 4α-methylsterols extracted from Messel oil shale

Nitrogen was bubbled through all solvents, reagents and reaction solutions before and during the oxidation. Standard chromium trioxide solution was prepared by dissolving chromium trioxide (2.67g) in conc. H_2SO_4 (2.3ml) and diluting with water to 10ml. The sample (*ca.* 15mg) was dissolved in acetone (2ml, distilled from potassium permanganate) and cooled (10-15 °C). Standard chromium trioxide solution (15ul) was added rapidly with stirring. After 2-5 minutes the reaction mixture was diluted with water (10ml) and extracted with dichloromethane (3 x 10ml). The organic phase was dried with pre-extracted anhydrous magnesium sulphate, filtered and evaporated to dryness under reduced pressure.

8.7.ii Reduction of 4α-methylsteroidal ketones extracted from Messel oil shale

The method described by Kupfler (1961) was followed. The sample (*ca.* 20 mg) was dissolved in dry isopropanol (10 ml) and sodium borohydride (2.5 mg) was added and stirred (15 hours). Water (20 ml) was added, followed by extraction with hexane-diethyl ether (1:1; 4 x 10 ml). The organic phase was dried with pre-extracted anhydrous magnesium sulphate, filtered and evaporated to dryness under reduced pressure.

8.7.iii Conversion of 4α-methylsterols extracted from Messel oil shale to 4α-methylsteroidal hydrocarbons

An aliquot of 4α-methylsterols (ca. 60mg), dissolved in ice-cold pyridine (1ml), was added to tosyl chloride (ca. 60mg; purified by Soxhlet extraction with hexane) and was left in the dark at room temperature for 4 days. The mixture was poured into ice-water (2ml) and a white precipitate formed, which was extracted into diethyl ether, washed with water, dilute HCl, water and dried. The ether was removed under reduced pressure to yield tosylates (46.8mg). The tosylates were heated under reflux (5 hours) in fresh dry diethyl ether (10ml) with $LiAlH_4$ (70mg). After cooling, water-HCl (1%) was slowly added and the product filtered. The product was extracted into diethyl ether and he ether removed under reduced pressure. The product was purified by alumina column chromatography, eluting with hexane.

REFERENCES

ALAM M., K.H.SCHRAM and S.M.RAY (1978). 24-Desmethyldinosterol: an unusual sterol from the dinoflagellate Gonyaulax diagensis. Tetrahedron Lett., 3517-3518.

ALAM M., G.E.MARTIN and S.M.RAY (1979). Dinoflagellate sterols II: Isolation and structure of 4-methylgorgostanol from the dinoflagellate Glenodinium foliaceum. J. Org. Chem., **44**, 4466-4467.

ALAM M., T.B.SANSING, J.R.GUERRA and A.D.HARMON (1981). Dinoflagellate sterols IV: Isolation and structure of 4α,23E,24E-trimethylcholestanol from the dinoflagellate Glenodinium hallii. Steroids, **38**, 375-382.

ALBRECHT P. (1969). Constituents organiques de roches sedimentaires.Ph.D. Thesis, Universite de Strasbourg, France.

ALBRECHT P. and G.OURISSON (1969). Triterpene alcohol isolated from oil shale. Science, **163**, 1192-1193.

ALLEN M.A. (1984). Study of 4α-methylsterols, stanols, sterones and stanones in a 50 m.y. old lake deposit. B.Sc. Thesis, Univ. Bristol, U.K.

ALLEN J.E., F.W.FORNEY and A.J.MARKOVETZ (1971). Microbial subterminal oxidation of alkanes and alk-1-enes. Lipids, **6**, 448-452.

BARNES M.A. and W.C.BARNES (1978). Organic compounds in lake sediments. In Lakes. Chemistry, Geology, Physics (Ed. A.Lerman), pp. 127-152, Springer-Verlag, Heidelberg.

BARNES M.A. and W.C.BARNES (1983). Oxic and anoxic diagenesis of diterpenes in lacustrine sediments. In Advances in Organic Geochemistry, 1981 (Eds. M.Bjoroy et al.), pp. 289-298, Wiley, Chichester.

BARRICK R.C., J.I.HEDGES and M.L.PETERSON (1980). Hydrocarbon geochemistry of the Puget Sound region-I. Sedimentary acyclic hydrocarbons. Geochim. Cosmochim. Acta, **44**, 1349-1362.

BEASTALL G.H., A.M.TYNDALL, H.H.REES and T.W.GOODWIN (1974). Sterols in Porphyridium species. 4α-Methyl-5α-cholesta-8,22-dien-3β-ol and 4α,24 -dimethyl-5α-cholesta-8,22-dien-3β-ol. Two novel sterols from Porphyridium cruentum. Eur. J. Biochem., **41**, 301-309.

BELCHER J.H., E.M.F.SWALE and J.HERON (1966). Ecological and morphological observations on a population of Cyclotella pseudostelligera Hustedt. J. Ecol., **54**, 335-340.

BERGMANN W. (1962). Sterols: their structure and distribution.

In Comparative Biochemistry, Vol. 3 (Eds. M.Florkin and H.Mason), pp. 103-162, Academic Press, New York.

BERTI G. and F.BOTTARI (1968). Constituents of ferns. In Progress in Phytochemistry (Vol. 1) (Eds. L.Reinheld and Y.Liwschitz), pp. 589-685, Interscience, New York.

BIRD C.W., J.M.LYNCH, S.J.PIRT, W.W.REID, C.J.W.BROOKS and B.S.MIDDLEDITCH (1971). Steroids and squalene in Methylococcus capsulatus grown on methane. Nature, 230, 473-474.

BLOCH K. (1976). In Reflections on Biochemistry (Eds. A.Kornberg, B.L.Honecker, L.Cornudella and L.Oro), pp. 143-150, Pergamon, New York.

BLUMER M. G.SOUZA and J.SASS (1970). Hydrocarbon pollution of edible shellfish by an oil spill. Mar. Biol., 5, 195-202.

BLUMER M., R.R.L.GUILLARD and T.CHASE (1971). Hydrocarbons of marine phytoplankton. Mar. Biol., **6**, 183-189.

BOON J.J., F.DE LANGE, P.J.W.SCHUYL, J.W.DE LEEUW and P.A.SCHENCK (1977). Organic geochemistry of Walvis Bay diatomaceous ooze. II. Occurrence and significance of the hydroxy fatty acids. In Advances in Organic Geochemistry, 1975 (Eds. R.Campos and J. Goni), pp. 255-273, Enadisma.

BOON J.J and J.W. DE LEEUW (1979). The analysis of wax esters, very long mid-chain ketones and steryl ethers isolated from Walvis Bay diatomaceous ooze. Marine Chem., **7**, 117-132.

BOON J.J., W.I.C.RIJPSTRA, F.DE LANGE, J.W.DE LEEUW, M.YOSHIOKA and Y.SHIMIZU (1979). Black Sea sterol - a molecular fossil for dinoflagellate blooms. Nature, 277, 125-127.

BOON J.J., W.I.C.RIJPSTRA, J.W. DE LEEUW and A.L.BURLINGAME (1980). The occurrence of triglycerides in Namibian Shelf diatomaceous ooze. Geochim. Cosmochim. Acta, **44**, 131-134.

BOUDOU J.-P., N.ROBINSON, S.C.BRASSELL and J.TRICHET (1984). Unpublished results.

BOURELLY P. (1968). Les algues d'eau douce. Initiation a la systematique. Tome III: Les algues bleues et rouges. Les Eugleniens, Peridiniens et Cryptomonadines (Ed. N.Boubee et Cie), Paris.

BOUVIER P., M.ROHMER, P.BEVENISTE and G.OURISSON (1976). $\Delta^{8(14)}$-Steroids in the bacterium Methylococcus capsulatus. Biochem. J., 159, 267-271.

BRADLEY W.H. (1966). Tropical lakes, copropel, and oil shale. Bull. Geol. Soc. Am., 77, 1333-1337.

BRADLEY W.H. (1970). Green River oil shale- concept of origin extended. Bull. Amer. Geol. Soc., **81**, 985-1000.

BRASSELL S.C. (1980). The lipids of deep-sea sediments: their origin and fate in the Japan Trench. Ph.D. Thesis, University of Bristol.

BRASSELL S.C., P.A.COMET, G.EGLINTON, P.J.ISAACSON, J.MCEVOY, J.R.MAXWELL, I.D.THOMSON, P.J.C.TIBBETTS and J.K.VOLKMAN (1980a). The origin and fate of lipids in the Japan Trench. In Advances in Organic Geochemistry, 1979 (Eds. A.G.Douglas and J.R.Maxwell), pp.375-392, Pergamon, Oxford.

BRASSELL S.C., A.P.GOWAR and G.EGLINTON (1980b). Computerised gas chromatography-mass spectrometry in analyses of sediments from the Deep Sea Drilling Project. In Advances in Organic Geochemistry, 1979 (Eds. A.G.Douglas and J.R.Maxwell), pp. 421-426, Pergamon, Oxford.

BRASSELL S.C., G.EGLINTON and J.R.MAXWELL (1981). Preliminary lipid analyses of 2 Quaternary sediments from the Middle America Trench, Southern Mexico transect, D.S.D.P. Leg 66. In Init. Rep. D.S.D.P., **66**, 557-580 (J.S.Watkins, J.C.Moore, et al.).

BRASSELL S.C. and G.EGLINTON (1983). Steroids and triterpenoids in deep sea sediments as environmental and diagenetic indicators. In Advances in Organic Geochemistry, 1981 (Eds. M.Bjoroy et al.), pp.684-697, Wiley, Chichester.

BRASSELL S.C., G.EGLINTON and J.R.MAXWELL (1983). The geochemistry of terpenoids and steroids. Biochem. Soc. Trans., **11**, 575-586.

BROOKS P.W. (1974). Isoprenoids and other lipids in Recent sediments. Ph.D. Thesis, Univ. Bristol, U.K.

BROOKS C.J.W., E.C.HORNING and J.S.YOUNG (1968). Characterisation of sterols by gas chromatography-mass spectrometry of the trimethylsilyl ethers. Lipids, **3**, 391-402.

BROOKS C.J.W., M.T.GILBERT and J.D.GIBERT (1973). New derivatives for gas-phase analytical resolution of enantiomeric alcohols and amines. Anal. Chem., **45**, 896-903.

BROOKS P.W. and J.R.MAXWELL (1974). Early stage fate of phytol in a recently deposited lacustrine sediment. In Advances in Organic Geochemistry, 1973 (Eds. B.Tissot and F.Bienner), pp. 977-991, Technip, Paris.

BROOKS P.W., G.EGLINTON, S.J.GASKELL, D.J.McHUGH, J.R.MAXWELL and R.P.PHILP (1976). Lipids of Recent sediments, Part I: Straight-chain hydrocarbons and carboxylic acids of some temperate lacustrine and sub-tropical lagoonal/tidal-flat sediments. Chem. Geol., **18**, 21-38.

BROOKS P.W., G.EGLINTON, S.J.GASKELL, D.J.McHUGH, J.R.MAXWELL and R.P.PHILP (1977). Lipids of Recent sediments, Part II: Branched and cyclic alkanes and alkanoic acids of some

temperate lacustrine and sub-tropical lagoonal/tidal-flat sediments. Chem. Geol., 20, 189-204.

BROOKS P.W., J.R.MAXWELL and R.L.PATIENCE (1978). Stereochemical relationships between phytol and phytanic acid, dihydrophytol and C_{18} ketone in recent sediments. Geochim. Cosmochim. Acta, 42, 1175-1180.

CARDOSO J.N., G.EGLINTON and P.J.HOLLOWAY (1977). The use of cutin acids in the recognition of a higher-plant contribution to Recent sediments. In Advances in Organic Geochemistry, 1975 (Eds. R.Campos and J.Goni), pp. 273-287, Enadisma, Madrid.

CARDOSO J.N. and G.EGLINTON (1983). The use of hydroxy acids as geochemical indicators. Geochim. Cosmochim. Acta, 47, 723-730.

CARDOSO J.N., S.J.GASKELL, M.M.QUIRK and G.EGLINTON (1983). Hydrocarbon and fatty acid distributions in Rostherne Lake sediment (England). Chem. Geol., 38, 107-128.

CHAPPE B., W.MICHAELIS and P.ALBRECHT (1980). Molecular fossils of Archaebacteria as selective degradation products of kerogen. In Advances in Organic geochemistry, 1979 (Eds. A.G.Douglas and J.R.Maxwell), pp. 265-274, Pergamon, Oxford.

CHICARELLI M.I. and J.N.CARDOSO (1983). The organic chemistry of the Paraiba Valley and Marau oil-shales. In Advances in Organic Geochemistry, 1981 (Eds. M.Bjoroy et al.), pp. 828-833, Pergamon, Oxford.

CHICARELLI M.I. and J.R.MAXWELL (1984). Naturally occurring, chlorophyll b related porphyrin. Tetrahedron Lett., 25, 4701-4704.

CHICARELLI M.I., L.P.DAMASCENO and J.N.CARDOSO (1984). Diagenetic chemistry of the Paraiba Valley oil shale. In Advances in Organic Geochemistry, 1983, in press.

COMET P.A., J.McEVOY, S.C.BRASSELL, G.EGLINTON, J.R.MAXWELL and I.D.THOMSON (1981). Lipids of an upper Albian Limestone, D.S.D.P. site 465, Section 465A-38-3. In Init. Rep. D.S.D.P., 62, 923-937 (J.Thiede, T.L.Vallier, et al.), Washington (U.S. Govt. Printing Office).

COOPER B.S. and D.G.MURCHISON (1969). Organic geochemistry of coal. In Organic Geochemistry (Eds. G.Eglinton and M.T.J.Murphy), pp. 699-726, Springer-Verlag, Heidelberg.

CORBET B., P.ALBRECHT and G.OURISSON (1980). Photochemical or photomimetic fossil triterpenoids in sediments and petroleums. J. Amer. Chem. Soc., 101, 1171-1173.

COX R.E., A.L.BURLINGAME, D.M.WILSON, G.EGLINTON and J.R.MAXWELL (1973). Botryococcene- a tetramethylated acyclic triterpenoid of algal origin. J. Chem. Soc., Chem. Commun., 284-285.

CRANWELL P.A. (1973). Chain-length distribution of n-alkanes from lake sediments in relation to post-glacial environmental change. Freshwat. Biol., 3, 259-265.

CRANWELL P.A. (1974). Monocarboxylic acids in lake sediments: Indicators, derived from terrestrial and aquatic biota, of palaeoenvironmental trophic levels. Chem. Geol., 14, 1-14.

CRANWELL P.A. (1976a). Decomposition of aquatic biota and sediment formation: organic compounds in detritus resulting from microbial attack on the alga Ceratium hirundinella. Freshwat. Biol., 6, 41-48.

CRANWELL P.A. (1976b). Decomposition of aquatic biota and sediment formation: lipid components of two blue-green algal species and of detritus resulting from microbial attack. Freshwat. Biol., 6, 481-488.

CRANWELL P.A. (1977). Organic geochemistry of Cam Loch (Sutherland) sediments. Chem. Geol., 20, 205-221.

CRANWELL P.A. (1978). Extractable and bound lipids in a freshwater sediment. Geochim. Cosmochim. Acta, 42, 1523-1532.

CRANWELL P.A. (1979). Decomposition of aquatic biota and sediment formation: bound lipids in algal detritus and lake sediments. Freshwat. Biol., 9, 305-313.

CRANWELL P.A. (1980). Branched/cyclic alkanols in lacustrine sediments (Great Britain): Recognition of iso- and anteiso-branching and stereochemical analysis of homologous alkan-2-ols. Chem. Geol., 30, 15-26.

CRANWELL P.A. (1981a). Diagenesis of free and bound lipids in terrestrial detritus deposited in a lacustrine sediment. Organic Geochem., 3, 79-89.

CRANWELL P.A. (1981b). The stereochemistry of 2- and 3-hydroxy fatty acids in a Recent lacustrine sediment. Geochim. Cosmochim. Acta, 45, 547-552.

CRANWELL P.A. (1982). Lipids of aquatic sediments and sedimenting particulates. Prog. Lipid Res., 21, 271-308.

CRANWELL P.A. (1983). Alkyl esters in recent sediments of two productive lakes. In Advances in Organic Geochemistry, 1981 (Eds. M.Bjoroy et al.), pp. 299-308, Pergamon, Oxford.

CRANWELL P.A. (1984). Alkyl esters, mid-chain ketones and fatty acids in late-glacial and post-glacial lacustrine sediments. In Advances in Organic Geochemistry, 1983, in press.

CRANWELL P.A. and J.K.VOLKMAN (1981). Alkyl and steryl esters in a recent lacustrine sediment. Chem. Geol., 32, 29-43.

DASTILLUNG M., P.ALBRECHT and G.OURISSON (1980a). Aliphatic and polycyclic alcohols in sediments. Hydroxylated derivatives of hopane and 3-methylhopane. J. Chem. Res., (S):168-169, (M):2353-2374.

DASTILLUNG M., P.ALBRECHT and G.OURISSON (1980b). Aliphatic and polycyclic ketones in sediments. C_{27}-C_{35} ketones and aldehydes of the hopane series. J. Chem. Res., (S): 166-167, (M): 2325-2352.

DAVIS J.B. (1968). Paraffinic hydrocarbons in the sulfate-reducing bacterium Desulfovibrio desulfuricans. Chem. Geol., 3, 155-160.

DAVISON W., J.HILTON, J.P.LISHMAN and W.PENNINGTON (1984). Contemporary lake transport processes determined from sedimentary records of copper mining. Env. Sci. Technol., in press.

DIDYK B.M., B.R.T.SIMONEIT, S.C.BRASSELL and G.EGLINTON (1978). Organic geochemical indicators of paleoenvironmental conditions of sedimentation. Nature, **272**, 216-222.

DJERASSI C. (1978). Recent advances in the mass spectrometry of steroids. Pure Appl. Chem, **50**, 171-184.

DJERASSI C. (1981). Recent studies in the marine sterol field. Pure Appl. Chem., 53, 873-890.

DODGE J.D. (1965). Chromosome structure in the dinoflagellates and the problem of the mesocaryotic cell. Progress, Int. Conf. on Protozoology, no. 2, London 1965, Abstracts Int. Congress Ser. 91, Abst. 339, pp.264-265, Excerpta Medica Foundation.

DODGE J.D. (1971). A dinoflagellate with both a mesocaryotic and a eucaryotic nucleus. I. Fine structure of the nuclei. Protoplasma, **73**, 145-157.

DODGE J.D. and R.M.CRAWFORD (1970). Fine structure of Ceratium hirundinella (Dinophyceae). J.Phycol., **6**, 137-149.

DRAGESCO J. (1965). Etude cytologique de quelques flagelles mesopsammiques. Cah. Biol. Mar., **6**, 83-115.

EDMUNDS K.L.H. (1982). Organic geochemistry of lipids and carotenoids in the Solar Lake microbial mat sequence. Ph.D. Thesis, Univ. Bristol, U.K.

EDMUNDS K.L.H., S.C.BRASSELL and G.EGLINTON (1980). The short-term diagenetic fate of 5α-cholestan-3β-ol: in situ radiolabelled incubation in algal mats. In Advances in Organic Geochemistry, 1979 (Eds. A.G.Douglas and J.R.Maxwell), pp. 427-434, Pergamon, Oxford.

EGLINTON G. and M.CALVIN (1967). Chemical fossils. Scientific American, **216**, 32-43.

EGLINTON G. and R.J.HAMILTON (1967). Leaf epicuticular waxes. Science, 156, 1322-1335.

EGLINTON G., D.H.HUNNEMAN and K.DOURAGHI-ZADEH (1968). Gas chromatographic-mass spectrometric studies of long chain hydroxy acids-II. The hydroxy acids and fatty acids of a 5000-yr.-old lacustrine sediment. Tetrahedron, 24, 5929-5941.

EGLINTON G., S.J.HAJIBRAHIM, J.R.MAXWELL, H.M.E.QUIRKE, G.J.SHAW, J.K.VOLKMAN and A.M.K.WARDROPER (1979). Lipids of aquatic sediments Recent and ancient. Phil. Trans. R. Soc., Ser. A, 293, 69-91.

ENSMINGER A. (1977). Evolution de composes polycycliques sedimentaires. Ph.D. Thesis, Universite Louis Pasteur, Strasbourg, France.

ENSMINGER A., P.ALBRECHT, G.OURISSON, B.J.KIMBLE, J.R.MAXWELL and G.EGLINTON (1972). Homohopane in the Messel oil shale: first identification of a C_{31} pentacyclic triterpane in Nature. Tetrahedron Lett., 36, 3861-3864.

ENSMINGER A., G.JOLY and P.ALBRECHT (1978). Rearranged steranes in sediments and crude oils. Tetrahedron Lett., 18, 1575-1578.

ERWIN J.A. (1973). Comparative biochemistry of fatty acids in eukaryotic microorganisms. In Lipids and Biomembranes of Eukaryotic Microorganisms (Ed. J.A.Erwin), pp. 41-143, Academic Press, London.

EVITT W.R. (1961). Observations on the morphology of fossil dinoflagellates. Micropaleontology, 7, 385-420.

FARRINGTON J.W. and J.G.QUINN (1971). Comparison of sampling and extraction techniques for fatty acids in recent sediments. Geochim. Cosmochim. Acta, 35, 735-741.

FARRINGTON J.W. and J.G.QUINN (1973). Biogeochemistry of fatty acids in recent sediments from Narrangansett Bay, Rhode Island. Geochim. Cosmochim. Acta, 37, 259-268.

FARRINGTON J.W., S.M.HENRICHS and R.andERSON (1977). Fatty acids from Buzzards Bay, Massachusetts. Geochim. Cosmochim. Acta, 41, 289-296.

FINLAY B.J., A.S.W.SPAN and J.M.P.HARMAN (1983). Nitrate respiration in primitive eukaryotes. Nature, 303, 333-336.

FOOKES C.J.R. (1983). Structure determination of nickel (II) deoxophylloerythrioetioporphyrin and a C_{30} homologue from an oil shale: Evidence that petroporphyrins are derived from chlorophyll. J. Chem. Soc., Chem. Commun., 1472-1473.

GAGOSIAN R.B. and J.W.FARRINGTON (1978). Sterenes in surface sediments from the southwest African shelf and slope. Geochim. Cosmochim. Acta, **42**, 1091-1101.

GAGOSIAN R.B. and S.O.SMITH (1979). Steroid ketones in surface sediments from the southwest African shelf. Nature, **277**, 287-289.

GAGOSIAN R.B., S.O.SMITH, C.LEE, J.W.FARRINGTON and N.M.FREW (1980). Steroid transformations in Recent marine sediments. In Advances in Organic Geochemistry, 1979 (A.G.Douglas and J.R.Maxwell), pp. 407-419, Pergamon, Oxford.

GALLEGOS R.J. (1971). Identification of new steranes, terpanes and branched paraffins in Green River Shale by combined capillary GC and MS. Anal. Chem., **43**, 1151-1161.

GASKELL S.J. and G.EGLINTON (1975). Rapid hydrogenation of sterols in a contemporary lacustrine sediment. Nature, **254**, 209-211.

GASKELL S.J. and G.EGLINTON (1976). Sterols of a contemporary lacustrine sediment. Geochim. Cosmochim. Acta, **40**, 1221-1228.

GELPI E., H.SCHNEIDER, J.MANN and J.ORO (1970). Hydrocarbons of geochemical significance in microscopic algae. Phytochemistry, **9**, 603-612.

GEORGE D.G. and S.I. HEANEY (1978). Factors influencing the spatial distribution of phytoplankton in a small productive lake. J. Ecol., **66**, 133-155.

GERRATH J.F. (1974). Two rare flagellates new to North America. Br. Phycol. J., **9**, 219.

GIESBRECHT P. (1962). Vergleichende Untersuchung an den Chromosen des Dinoflagellaten Amphidinium elegans und denen der Bakterien. Zentralbl. Bakteriol. Parasitenkd., Infektionskr. Hyg. L. Orig., **187**, 452-498.

GIGER W. and C.SCHAFFNER (1977). Aliphatic, olefinic, and aromatic hydrocarbons in Recent sediments of a highly eutrophic lake. In Advances in Organic Geochemistry, 1975 (Eds. R.Campos and J.Goni), pp. 375-390, Enadisma, Madrid.

GIGER W., C.SCHAFFNER and S.G.WAKEHAM (1980). Aliphatic and olefinic hydrocarbons in recent sediments of Greifensee, Switzerland. Geochim. Cosmochim. Acta, **44**, 119-129.

GILLAN F.T., R.B.JOHNS, T.V.VERHEYEN, P.D.NICHOLS, R.J.ESDAILE and H.J.BAVOR (1983). Monounsaturated fatty acids as specific bacterial markers in marine sediments. In Advances in Organic Geochemistry, 1981 (Eds. M.Bjøroy et al.), pp. 198-206, Wiley, Chichester.

GREINER A.Ch., C.SPYCKERELLE and P.ALBRECHT (1976). Aromatic

hydrocarbons from geological sources. I. New naturally occurring phenanthrene and chrysene derivatives. Tetrahedron, 32, 257-260.

GREINER A.Ch., C.SPYCKERELLE, P.ALBRECHT and G.OURISSON (1977). Aromatic hydrocarbons from geclogical sources. V. Monc and diaromatic hopane derivatives. J. Chem. Res., (M): 3829-3871; (S): 334-335.

HABERMEHL G. and H.J.HUNDRIESER (1983). Fossil relics of water bloom. Naturwissenschaften, 70, 566-568.

HABERMEHL G. and G.SPRINGER (1983). Lankettige diole im Messeler Olschiefer. Naturwissenschaften, 70, 197-198.

HAN J., E.D.McCARTHY, W.VAN HOEVEN, M.CALVIN and W.H.BRADLEY (1968). Organic geochemical studies. II. A preliminary report on the distribution of aliphatic hydrocarbons in algae, in bacteria, and in recent lake sediment. Proc. Nat. Acad. Sci., U.S.A., 59, 29-33.

HAN J. and M.CALVIN (1969). Hydrocarbon distribution of algae and bacteria, and microbiological activity in sediments. Proc. Nat. Acad. Sci., U.S.A., 64, 436-443.

HARRINGTON G.W., D.H. BEACH, J.E. DUNKAM and G.G. HOLZ Jr. (1970). Marine photosynthetic and heterctrophic spp., polyunsaturated fatty acid composition. Protozool., 17, 213-219.

HAWORTH E.Y. (1984). Stratigraphic changes in algal remains (diatoms and chrysophytes) in the recent sediments of Blelham Tarn, English Lake District. In Lake Sediments and Environmental History (Eds. E.Y.Haworth and J.W.G.Lund), pp. 69-92, Leicester University Press.

HITCHCCCK C. and A.ROSE (1971). The stereochemistry of α-oxidation of fatty acids in plants: the configuration of biosynthetic long-chain 2-hydroxy acids. Biochem. J., 125, 1155-1156.

HOFFMANN C.F. (1984). Synthesis and geochemical applications of aromatic steroid hydrocarbons. Ph.D. Thesis, Univ. Bristol, U.K.

HOLLand E.G. (1981). Coniston copper mines, a field guide, Cicerone Press, Milnthorpe.

HOLLOWAY P.J. (1973). Cutins of Malus pumila fruits and leaves. Phycchemistry, 12, 2913-2920.

HOWARD D.L. (1980). Polycyclic triterpenes of the anaerobic photosynthetic bacterium Rhodomicrobium vannielli. Ph.D. Thesis, Univ. of California, Los Angeles.

HUANG W.-Y. and W.G.MEINSCHEIN (1976). Sterols as source indicators of organic materials in sediments. Geochim.

Cosmochim. Acta, **40**, 323-330.

HUSSLER G. and P.A. ALBRECHT (1983). C_{27} - C_{29} monoaromatic anthrasteroid hydrocarbons in Cretaceous Black shales. Nature, **304**, 262-263.

HUSSLER G., B. CHAPPE, P. WEHRUNG and P. ALBRECHT (1981). C_{27} - C_{29} ring A monoaromatic steroids in Cretaceous black shales. Nature, **294**, 556-558.

ISHIWATARI R. (1976). Normal alkane distribution in a 200-meter core sample from Lake Biwa - A preliminary report. In: Paleolimnology of Lake Biwa and the Japanese Pleistocene, Vol. 4 (Ed. S. Horie), pp. 240-246.

ISHIWATARI R. (1976). Fatty acids and perylene in a 200-meter core sample from Lake Biwa - A discussion. (Ibid.), pp. 246-253.

ISHIWATARI R. and K. KAWAMURA (1977). Variation of fatty acid composition in a sediment of Lake Biwa. In: Paleolimnology of Lake Biwa and the Japanese Pleistocene, Vol. 5 (Ed. S. Horie), pp. 181-194.

ISHIWATARI R., K. OGURA and S. HORIE (1977). Characterisation of organic compounds in a sediment of Lake Haruna - I. Fatty acids. In: Paleolimnology of Lake Biwa and the Japanese Pleistocene, Vol. 5 (Ed. S. Horie), pp. 221-233.

ISHIWATARI R., K.OGURA and S.HORIE (1980). Organic geochemistry of a lacustrine sediment (Lake Haruna, Japan). Chem. Geol., **29**, 261-280.

ITOH T., D.SICA and C.DJERASSI (1983). Minor and trace sterols in marine invertebrates: part 35. Isolation and structure elucidation of 74 sterols from the sponge Axinella cannabina. J. Chem. Soc. Perkin Trans., 1, 147-153.

JACOB J. (1975). Waxes containing secondary alcohols from the uropygial gland secretion of Nycticorax nycticorax (night heron). Z. Physical Chem., **356**, 1823-1825.

JAVOR B., S.C.BRASSELL and G.EGLINTON (1979). A laboratory/field method for radiolabelled incubation studies in algal-bacterial mats and other microbial ecosystems. Oceanol. Acta, 2, 19-22.

JEFFREY S.W., M.SIELICKI and F.T.HAXO (1975). Chloroplast pigment patterns in dinoflagellates. J. Phycol., 11, 374-384.

JONES G.J., P.D.NICHOLS and R.B.JOHNS (1983). The lipid composition of Thoracosphaera heimii: evidence for inclusion in the Dinophyceae. J. Phycol., **19**, 416-420.

JONES J.G., M.J.L.G. ORLANDI and B.M. SIMON (1979). A microbiological study of sediments from the Cumbrian lakes.

J. Gen. Microbiol., **115**, 37-48.

KAWAMURA K., R.ISHIWATARI and M.YAMAZAKI (1980). Identification of polyunsaturated fatty acids in surface lacustrine sediments. Chem. Geol., **28**, 31-39.

KIMBLE B.J., J.R.MAXWELL, R.P.PHILP and G.EGLINTON (1974a). Tri- and tetraterpenoid hydrocarbons in the Messel oil shale. Geochim. Cosmochim. Acta, **38**, 1165-1181.

KIMBLE B.J., J.R.MAXWELL, R.P.PHILP and G.EGLINTON (1974b). Identification of steranes and triterpanes in geolipid extracts by high-resolution gas chromatography and mass spectrometry. Chem. Geol., **14**, 173-198.

KLOK J. (1984). Composition and origin of complex organic matter in recent marine sediments. Significance of bacterial biomarkers. Ph.D Thesis, Univ. Delft, Netherlands.

KNUDSEN E., E.JANTZEN, K.BRYN, J.G.ORMEROD and R.SIREVAG (1982). Quantitative and structural characteristics of lipids in Chlorobium and Chloroflexus. Arch. Microbiol., **132**, 149-154.

KOBAYASHI M., A.TOMICKA and H.MITSUHASHI (1979). Isolation of 23-methylcholesta-5,22-dien-3B-ol from the soft coral Sarcophyton glaucum. Chem. Pharm. Bull., **27**, 1951-1953.

KOKKE W.C.M.C., N. WITHERS, B.J.MASSEY, W.FENICAL and C.DJERASSI (1979). Isolation and synthesis of 23-methyl-22-dehydrocholesterol - a marine sterol of biosynthetic significance.Tetrahedron Lett,, 3601-3604.

KOKKE W.C.M.C., W.FENICAL and C.DJERASSI (1981a). Sterols with unusual nuclear unsaturation from three cultured marine dinoflagellates. Phytochemistry, **20**, 127-134.

KOKKE W.C.M.C., W.FENICAL, L.BOHLIN and C.DJERASSI (1981b). Sterol synthesis by cultured zooxanthellae; implications concerning sterol metabolism in the host-symbiont association in Caribean gorgonians.Comp. Biochem. Physiol., **68B**, 281-287.

KOKKE W.C.M.C., W.FENICAL and C.DJERASSI (1982). Steroids of the cultured dinoflagellate Pyrocystis lunula. Steroids, **40**, 307-318.

KOENIGSWALD W. VON (1980). Fossillagerstatte Messel - Literaturubersicht de Forschungergebnisse aus den Jahren 1969-1979. Geol. B. Hessen, **108**, 23-38.

KOLATTUKUDY P.E.(1976). Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam.

KOZHOVA O.M. (1959). O podlednom "tsvetenii" ozera Baykal (On the water bloom under the ice in Lake Baikal). Bot. Zh. SSSR, **44**, 1001-1004.

KUPFER D. (1961). Altered selectivity of reduction of steroidal carbonyls. Tetrahedron, 15, 193-196.

LAFLAMME R.E.and R.A.HITES (1978). The global distribution of polycyclic aromatic hydrocarbons in recent sediments. Geochim. Cosmochim. Acta, **42**, 289-303.

LASETER J.L. and J.D.WEETE (1971). Fatty acid ethyl esters of Rhizopus arrhizus. Science, **172**, 864-865.

LEEUW J.W. DE, W.I.C.RIJPSTRA and P.A.SCHENCK (1981). The occurrence and identification of C_{30}, C_{31} and C_{32} alkan-1,15-diols and alkan-15-one-1-ols in Unit I and Unit II Black Sea sediments. Geochim. Cosmochim. Acta, **45**, 2281-2285.

LEEUW J.W. DE, W.I.C.RIJPSTRA, P.A.SCHENCK and J.K.VOLKMAN (1983). Free, esterified and residual bound sterols in Black Sea Unit I sediments. Geochim. Cosmochim. Acta, **47**, 455-465.

LEO R.F. and P.L. PARKER (1966). Branched-chain fatty acids in sediments. Science, **152**, 649-650.

LIVINGSTONE D. (1979). Algal remains in recent lake sediments. Ph.D Thesis, Univ. of Leicester.

LIVINGSTONE D. and R.S. CAMBRAY (1978). Confirmation of ^{137}Cs dating by algal stratigraphy in Rostherne Mere. Nature, **276**, 259-261.

LOEBLICH A.R.III (1976). Dinoflagellate evolution: speculation and evidence. J. Protozool., **23**, 13-28.

MACKENZIE A.S., S.C.BRASSELL, G.EGLINTON and J.R.MAXWELL (1982). Chemical fossils: The geological fate of steroids. Science, **217**, 491-504.

McEVOY J. (1983). The origin and diagenesis of organic lipids in sediments from the San Miguel Gap. Ph.D. Thesis, Univ. of Bristol.

MCLEAN J., G.H.RETTIE and F.S.SPRING (1958). Triterpenoids from peat. Chem. Ind. (London), 1515.

MACKERETH F.J.H. (1969). A short core sampler for subaqueous deposits. Limnol. Oceanog., **14**, 145-151.

MALLORY F.B., J.T.GORDON and J.L.CONNER (1963). The isolation of a pentacyclic triterpenoid alcohol from a protozoan. J. Amer. Chem. Soc., **85**, 1362-1363.

MANGONI L., D. MEROLA, P. MONACO, M. PAVILLI and L. PREVITERA (1984). A biogenetically new tetraterpene alcohol from Elodea canadensis. Tetrahedron Lett., **25**, 2597-2600.

MATSUO A., M.NAKYAMA, S.HAYASHI and K.NAGAI (1980). Fatty acid ethyl esters in the liverwort Conocephalum conicum.

Phytochemistry, **19**, 1848-1849.

MATTERN G., P.ALBRECHT and G.OURISSON (1970). 4-Methylsterols and sterols in Messel Shale (Eocene). Chem. Commun., 1570-1571.

MATTHES G. (1956). Ein Beitrag zur Geologie des Olschieferrorkommers von Messel bei Darmstadt. Jahrester. Mitt. oberhein. geol. Ver., **38**, 11-21.

MATTHES G. (1968). Les couches Eocenes dans la region du foss rhenan septentrional. Mem. Bureau Rech. Geol. Min., **58**, 327-337.

MAXWELL J.R., R.E.COX, R.G.ACKMAN and S.N.HOOPER (1972). The diagenesis and maturation of phytol. The stereochemistry of 2,6,10,14-trimethylpentadecane from an ancient sediment. In Advances in Organic Geochemistry, 1971 (Eds. H.R. von Gaertner and H.Wehner), pp. 277-291, Pergamon, Oxford.

MAXWELL J.R., A.S.MACKENZIE and J.K.VOLKMAN (1980). Configuration at C-24 in steranes and sterols. Nature, **286**, 694-697.

MAXWELL J.R. and A.M.K.WARDROPER (1982). Early transformations of isoprenoid compounds in surface sedimentary environments. In Sediment Microbiology (Eds. D.B.Nedwell and C.M.Brown), pp. 203-229, Academic Press, London.

MAZUR V., A.WEIZMANN and F.SONDHEIMER (1958). Steroids and triterpenoids of citrus fruit. III. The structure of citrostadienol, a natural 4α-methylsterol. J. Amer. Chem. Soc., **80**, 6293-6296.

MERMOUD F., F.O.GULACAR, S.SILES, B.CHASSAING and A.BUCHS (1982). 4-Methylstanols in recent lacustrine sediments: terrestrial, plankton or some other origin? Chemosphere, **11**, 557-567.

MEYERS P.A., M.J.LEENHEER, B.J.EADIE and S.J.MAULE (1984). Organic geochemistry of suspended and settling particulate matter in Lake Michigan. Geochim. Cosmochim. Acta, **48**, 443-452.

MEYERS P.A., D.R. WHITEHEAD and O.E. KAWKA (1984). Geolipid, pollen, and diatom stratigraphy of postglacial lacustrine sediments. In Advances in Organic Geochemistry, 1983, in press.

MINALE and G.SODANO (1976). In Marine Natural Products Chemistry (Eds. D.J.Faulkner and W.H.Fenical), pp.87-109, Plenum, New York.

MORRISON R.I. and W. BRICK (1967). The wax fraction of soils: separation and determination of some components. J. Sci. Fd. Agric., **18**, 351-355.

MULLER O.F. (1773). Vemium terrestrium et fluviatilum seu animalium infusoriorum, helminthicorum et testaceorum, non marinorum secinta historia. Heineck et Faber typis Martini

Hallager, Havniae et Lipsiae 1(1).

NACCARATO W.F., R.A.GELMAN, J.C.KAWALEK and J.R.GILBERTSON (1972). Characterisation and metabolism of free fatty alcohols from Escherichia coli. Lipids, 7, 275-281.

NEAL A.C. (1984). The biogeochemical significance of copepod feeding. Ph.D. Thesis, Univ. of Bristol.

NEFF J.M. (1979). Polycyclic aromatic hydrocarbons in the aquatic environment. Applied Science, London.

NES W.R. and M.L.MCKEAN (1977). Biochemistry of Steroids and Other Isopentoids, Univ. Park Press, Baltimore.

NICHOLS P.D., G.J.JONES, J.W. DE LEEUW and R.B.JOHNS (1984). The fatty acid and sterol ccmposition of two marine dinoflagellates. Phytochemistry, **23**, 1043-1047.

NISHIMIURA M. (1977). The geochemical significance in early sedimentation of geolipids obtained by saponification of lacustrine sediments. Geochim. Cosmochim. Acta, **41**, 1817-1823.

NISHIMURA M. (1978). Geochemical characteristics of the reduction zone of sterols in Suwa sediments and environmental factors controlling the conversion of stenols into stanols. Geochichim. Cosmochim. Acta, **42**, 349-357.

NISHIMURA M. and T.KOYAMA (1977). The occurrence of stanols in various organisms and the behaviour of sterols in contemporary sediments. Geochim. Cosmochim. Acta, **41**, 379-385.

OGURA K. and T.HANYA (1973). Cholestanol-cholesterol ratio in a 200 metre core sample of Lake Biwa. Proc. Jap. Acad., **49**, 201-204.

OURISSON G., P.ALBRECHT and M.ROHMER (1979). The hopanoids. Palaeochemistry and biochemistry of a group of natural products. Pure Appl. Chem., **51**, 709-729.

PARKER P.L. (1969). Fatty acids and alcohols. In Organic Geochemistry (Eds. G.Eglinton and M.T.J.Murphy), pp. 357-373, Springer-Verlag, Heidelberg.

PATTERSON G.W. (1967). Sterols of Chlorella II. The occurrence of an unusual sterol mixture in Chlorella vulgaris. Plant Physiol., **42**, 1457-1459.

PATTERSON G.W. (1974). Sterols of some green algae. Comp. Biochem. Physiol., **47b**, 453-457.

PEAKMAN T.M., N.A.LAMB and J.R.MAXWELL (1984). Naturally occurring spiro steroid hydrocarbons. Tetrahedron Lett., **25**, 349-352.

PEARSALL W.H. (1921). The development of vegetation in English Lakes considered in relation to the general evolution of glacial lakes and rock basins. Proc. Roy. Soc. London, **B92**, 259-284.

PENNINGTON W. (1981). Records of a lake's life in time: the sediments. Hydrobiologia, **79**, 197-219.

PENNINGTON W., E.Y.HAWORTH, A.P.BONNY and J.P.LISHMAN (1972). Lake sediments in Northern Scotland. Phil. Trans. Roy. Soc. London B., **264**, 191-294.

PERRY G.J., J.K. VOLKMAN, R.B. JOHNS and H.J. BAVOR (1979). Fatty acids of bacterial origin in contemporary marine sediments. Geochim. Cosmochim. Acta, **43**, 1715-1725.

PHILP R.P., J.R.MAXWELL and G.EGLINTON (1976). Environmental organic geochemistry of aquatic sediments. Sci. Prog., Oxf., **63**, 521-545.

PRAHL F.G., G.EGLINTON , E.D.S. CORNER, S.C.M. O'HARA and T.E.V. FORSBERG (1984). Changes in plant lipids during passage through the gut of Calanus. J. Mar. Biol. Ass. U.K., **64**, 317-334.

POLLINGHER U. (1978). Peridinium cinctum fa. westii (Lemm.) Lef.: Morphology and systematics. In Lake Kinneret (Ed. C.Serruya), pp. 259-267, Dr.W.Junk bv, The Hague.

QUIRK M.M. (1978). Lipids of peat and lake environments. Ph.D. Thesis, Univ. of Bristol, U.K.

QUIRK M.M., A.M.K.WARDROPER, P.W.BROOKS, R.E.WHEATLEY and J.R.MAXWELL (1980). Transformations of acyclic and cyclic isoprenoids in recent sedimentary environments. Presented at Colloques Internationaux du CNRS no. 293 Biogochimie de la Matiere Organique a L'Interface Eau-Sediment Marin.

RAPOPORT H. and H.P.HAMLOW (1981). Chlorobium Chlorophyll-660. The esterifying alcohol. Biochem. Biophys. Res. Commun., **6**, 134-137.

RHEAD M.M., G.EGLINTON, G.H. DRAFFAN and P.J. ENGLAND (1971). Conversion of oleic acid to saturated fatty acids in Severn estuary sediments. Nature, **232**, 327-330.

RILEY J.P. and R.CHESTER (1971). Introduction to Marine Chemistry, Academic Press, London.

RIZZO P.J. and L.D.NOODEN (1973). Isolation and chemical composition of dinoflagellate nuclei. J. Protozool., **20**, 666-672.

ROBINSON N., G.EGLINTON, S.C.BRASSELL and P.A.CRANWELL (1984a). Dinoflagellate origin for sedimentary 4α-methylsteroids and 5α(H)-stanols. Nature, **308**, 439-441.

ROBINSON N., P.A.CRANWELL, B.J.FINLAY and G.EGLINTON (1984b). Lipids of aquatic organisms as potential contributors to lacustrine sediments. In Advances in Organic Geochemistry, 1983 (in press).

ROHMER M., W.C.M.C.KCKKE, W.FENICAL and C.DJERASSI (1980a). Isolation of 2 new C_{30} sterols, (24E)-24-N-propylidene-cholesterol and 24ξ-N-propylcholesterol, from a cultured marine Chrysophyte. Steroids, **35**, 219-231.

ROHMER M., M.DASTILLUNG and G.OURISSON (1980B). Hopanoids from C_{30} to C_{35} in recent muds. Naturwissenschaften, **67**, 456-458.

RUBINSTEIN I. and P.ALBRECHT (1975). The occurrence of nuclear methylated steranes in a shale. Chem. Commun., 957-958.

RYHAGE R. and E.STENHAGEN (1965). Mass spectrometric studies XI. On the ions of m/e 84 + 14n present in mass spectra of dibasic acids. Arkiv. Kemi, **23**, 167-175.

SARGENT J.R. (1976). The structure, metabolism and function of lipids in marine organisms. Biophys. Perspect. Mar. Biol., 3, 149-212.

SCHWENDINGER R.B. and J.G. ERDMAN (1964). Sterols in recent aquatic sediments. Science, **144**, 1575-1576.

SERK-HANSSEN K., S.STALLBERG-STENHAGEN and E.STENHAGEN (1953). Optically active higher aliphatic hydroxy-compounds. 1. Ark. Kemi, 5, 203-221.

SERRUYA C. (1978). Lake Kinneret (Ed. C.Serruya), Dr.W.Junk bv, The Hague.

SHIMIZU Y., M.ALAM and A.K.KOBAYASHI (1976). Dinosterol, the major sterol with a unique side chain in the toxic dinoflagellate, Gonyaulax tamarensis. J. Amer. Chem. Soc., **98**, 1059-1060.

SIMONEIT B.R.T. (1978). The organic chemistry of marine sediments. In: Chemical Oceanography (Eds. J.P. RILEY and R. CHESTER), pp. 233-311, Academic Press, New york.

SIMONEIT B.R.T., H.I.HALPERN and B.M.DIDYK (1980). Lipid productivity of a high Andean Lake. In Biogeochemistry of Ancient and Modern Environments (Eds. P.A.Trudinger, M.R.Walter and B.J.Ralph), pp. 201-209, Australian Academy of Science, Canberra.

SITTLER C. (1968). L'analyse pollinique dans l'Est de la France. Etude des formations Eocènes ou rapportées a l'Eocène et des stratolypes palynologiques de Borken et de Messel. Mem. Bur. Rech. Geol. Min., **58**, 165-171.

SMITH A.G. and L.J.GOAD (1975). Conversion of cholest-5-en-3β-ol into cholest-7-en-3β-ol by the echinoderm Asterias rubens and Solaster papposus. Biochem. J., **146**, 35-40.

SMITH D.J., G.EGLINTON, R.J.MORRIS and E.L.POUTANEN (1983). Aspects of the steroid geochemistry of an interfacial sediment from the Peruvian upwelling. Oceanol. Acta, **6**, 211-220.

SMITH D.J., G.EGLINTON and R.J.MORRI (1983). Occurrence of long-chain alkan-diols and alkan-15-one-1-ols in a Quaternary Sapropel from the Eastern Mediterranean. Lipids, **18**, 902-905.

SOYER M.O. (1969). Rapports existant entre chromosomes et membranes nucleaire chez un dinoflagelle parasite du genre Blastodinium Chatton. C.R. Acad. Sci. Paris, **268d**, 2082-2084.

SPYCKERELLE C., A.Ch.GREINER, P.ALBRECHT and G.OURISSON (1977a). Aromatic hydrocarbons from geological sources. III. A tetrahydrochrysene derived from triterpenes, in Recent and old sediments: 3,3,7-trimethyl-1,2,3,4-tetrahydrochrysene. J. Chem. Res., (M):3746-3777; (S):330-331.

SPYCKERELLE C., A.Ch.GREINER, P.ALBRECHT and G.CURISSON (1977b). Aromatic hydrocarbons from geological scurces. IV. An octahydrochrysene derived from triterpenes in Recent and old sediments, 3,3,7,12a-tetramethyl-1,2,3,4,4a,11,12,12a-octahydrochrysene. J. Chem. Res., (M):3801-3828; (S): 332-333.

STARMACH K. (1974). Flora Slodkowodna Polski Vol. 4, pp. 326-329, State Scientific Publishers, Krakow.

STOSCH H.A.VON (1973). Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, Gymnodinium pseudopalustre Schiller and Woloszynskia apiculata sp. nov. Br. Phycol. J., **8**, 105-134.

STRAIN H.H., W.A.SVEC, K.AIZETMULLER, M.C.GRandOLFO, T.T.KATZ, H.KJOSEN, S.NORGARD, S.LIAAEN-JENSEN, F.T.HAXO, P.WEGFAHRT and H.RAPOPORT (1971). The structure of peridinin, the characteristic dinoflagellate carotenoid. J. Amer. Chem. Soc., **93**, 1823-1824.

STRANSKY K., M.STREIBL and V.KUBELKA (1970). Natural waxes. XV. Hydrocarbcn constituents of the leaf wax from the walnut tree (Juglans regia). Collect. Czech. Chem. Commun., **35**, 882-891.

SWENSON W., B.TAGLE, L.CLARDY, N.W.WITHERS, W.C.M.C.KOKKE, W.H.FENICAL and C.DJERASSI (1980). Peridinosterol- a new Δ^{17}-unsaturated sterol from two cultured marine algae.

SYKES P.J. (1961). In A Guidebook to Mechanism in Organic Chemistry, p. 90, Longman, London.

TAN Y.L. and M.HEIT (1981). Biogenic and abiogenic polynuclear aromatic hydrocarbons in sediments from two remote Adirondack lakes. Geochim. Cosmochim Acta, **45**, 2267-2279.

TAPPAN H. (1980). The Paleobiology of Plant Protists, Freeman,

San Francisco.

TAYLOR J., A.M.K.WARDROPER and J.R.MAXWELL (1980). Extended hopane derivatives in sediments: identification by ^{1}H NMR. Tetrahedron Lett., **21**, 655-656.

TESHIMA S., A.KANAZAWA and A.TAGO (1980). Sterols of the dinoflagellate, Noctiluca milialis. Mem. Fac. Fish., Kagoshima Univ., **29**, 319-326.

TESHIMA S., A.KANAZAWA, N.KAMEZAKI and H.HIRATA (1979). Sterols of the rotifer. Bull. Jap. Soc. Fish., **45**, 1495-1501.

THOMSON I.D., S.C.BRASSELL, G.EGLINTON and J.R.MAXWELL (1982). Preliminary lipid analysis of section 481-2-2. In J.R.Curray, D.G.Moore et al. Init. Rep. D.S.D.P., **64**, pp. 913-919, Washington (U.S. Govt. Printing Office).

TISSOT B.P. and D.H. WELTE (1978). Petroleum Formation and Occurrence, Spring-Verlag, Berlin.

TULLOCH A.P. (1976). Chemistry of waxes of higher plants. In Chemistry and Biochemistry of Natural Waxes (Ed. P.E.Kolattukudy), pp. 235-287, Elsevier, Amsterdam.

VAN GRAAS G., F. DE LANGE, J.W. DE LEEUW and P.A. SCHENCK (1982a). A-nor-steranes, a novel class of sedimentary hydrocarbons. Nature, **296**, 59-61.

VAN GRAAS G., F. DE LANGE, J.W. DE LEEUW and P.A. SCHENCK (1982b). De-A-steroid ketones and de-A-steroid hydrocarbons in shale indicate a novel diagenetic pathway. Nature, **299**, 437-439.

VAN VLEET E.S. and J.G. QUINN (1979). Early diagenesis of fatty acids and isoprenoid alcohols in estuarine and coastal sediments. Geochim. Cosmochim. Acta, **43**, 289-303.

VOLKMAN J.K. and R.B. JOHNS (1980). Sterols and fatty acids of the marine diatom Biddulphia sinensis. Phytochemistry, **19**, 1809-1813.

VOLKMAN J.K., R.B.JOHNS, F.T.GILLAN, G.J.PERRY and H.J.BAVOR (1980). Microbial lipids of an intertidal sediment: I. Fatty acids and hydrocarbons. Geochim. Cosmochim. Acta, **44**, 1133-1143.

VOLKMAN, J.K., D.J.SMITH, G.EGLINTON, T.E.V.FORSBERG and E.D.S.CORNER (1981). Sterol and fatty acid composition of 4 marine haptophycean algae. J. Mar. Biol. Ass.,U.K., **61**, 509-527.

VOLKMAN J.K., J.W.FARRINGTON, R.B.GAGOSIAN and S.G.WAKEHAM (1983). Lipid composition of coastal marine sediments from the Peru upwelling region. In Advances in Organic Geochemistry, 1981 (Eds. M.Bjøroy et al.), pp. 228-240.

VOLKMAN J.K., R.B.GAGOSIAN and S.G.WAKEHAM (1984). Free and esterified sterols of the marine dinoflagellate, Gonyaulax polygramma. Lipids, **19**, 457-465.

WAKEHAM S.G. (1982). Sources and fates of organic matter from a sediment trap experiment in the equatorial north Atlantic: wax esters, steryl esters, triacylglycerols and alkyldiacylglycerols. Geochim. Cosmochim. Acta, **46**, 2239-2257.

WAKEHAM S.G., C.SCHAFFNER and W.GIGER (1980a). Diagenetic polycyclic aromatic hydrocarbons in Recent sediments: Structural information obtained by high performance liquid chromatography. In Advances in Organic Geochemistry, 1979 (Eds. A.G.Douglas and J.R.Maxwell), pp. 353-363, Pergamon, Oxford.

WAKEHAM S.G., C.SCHAFFNER and W.GIGER (1980b). Polycyclic aromatic hydrocarbons in Recent lake sediments: II. Compounds derived from biogenic precursors during early diagenesis. Geochim. Cosmochichim. Acta, **44**, 415-429.

WAKEHAM S.G., J.W.FARRINGTON, R.B.GAGOSIAN, C.LEE, H.DEBARR, G.E.NIGRELLI, B.W.TRIPP, S.C.SMITH and N.M.FREW (1980c). Organic matter fluxes from sediment traps in the equatorial Atlantic Ocean. Nature, **286**, 798-800.

WAKEHAM S.G. and N.M.FREW (1983). High temperature glass capillary gas chromatography/mass spectrometry of wax esters, steryl esters and triglycerides. Lipids, **17**, 831-843.

WARDROPER A.M.K. (1979). Aspects of the geochemistry of polycyclic isoprenoids. Ph.D. Thesis, Univ. of Bristol.

WARDROPER A.M.K., P.W.BROOKS, M.J.HUMBERTSON and J.R.MAXWELL (1977). Analysis of steranes and triterpanes in geolipid extracts by automatic classification of mass spectra. Geochim. Cosmochim. Acta, **41**, 499-510.

WETTSTEIN-KNOWLES P. VON and A.G.NETTING (1976). Esterified alkan-1-ols and alkan-2-ols in barley epicuticular wax. Lipids, **11**, 478-484.

WHEELER M.A. and C.E.HOLMLUND (1975). Wax esters isolated from Tetrahymena pyriformis. Lipids, **10**, 260-262.

WILLIAMS B.L., L.J.GOAD and T.W.GOODWIN (1967). The sterols of grapefruit peel. Phytochemistry, **6**, 1137-1145.

WITHERS N., (1983). Dinoflagellate sterols. In Marine Natural Products (Ed. P.J.Scheuer), pp. 87-130, Academic Press, New York.

WITHERS N., and J.C.NEVENZEL (1977). Phytyl esters in a marine dinoflagellate. Lipids, **12**, 989-993.

WITHERS N., R.C.TUTTLE, G.G.HOLZ, D.H.BEACH, L.J.GOAD and

T.W.GOODWIN (1978). Dehydrodinosterol, dinosterone and related sterols of a non-photosynthetic dinoflagellate, Crypthecodinium cohnii. Phytochemistry, **17**, 1987-1989.

WITHERS N., W.C.M.C.KOKKE, M.ROHMER, W.H.FENICAL and C.DJERASSI (1979a). Isolation of sterols with cyclopropyl-containing side-chains from the cultured marine alga Peridinium foliaceum. Tetrahedron Lett., **38**, 3605-3608.

WITHERS N., R.C.TUTTLE, L.J.GOAD and T.W.GOODWIN (1979b). Dinosterol side-chain biosynthesis in a marine dinoflagellate, Crypthecodinium cohnii. Phytochemistry, **18**, 71-73.

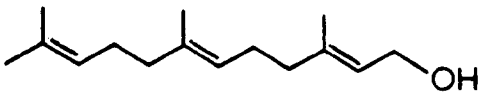
WITHERS N., R.C.TUTTLE, L.J.GOAD and T.W.GOODWIN (1979c). A new sterol, 4α-methyl-5α-cholest-8(14),24(28)-dien-3β-ol, from the marine dinoflagellate Amphidinium carterae. Phytochemistry, **18**, 899-901.

WITHERS N., W.C.M.C.KOKKE, W.FENICAL and C.DJERASSI (1982). Sterol patterns of cultured zooxanthellae isolated from marine invertebrates: Synthesis of gorgosterol and 23-desmethylgorgosterol by aposymbictic algae. Proc. Natl. Acad. Sci. U.S.A., **79**, 3764-3768.

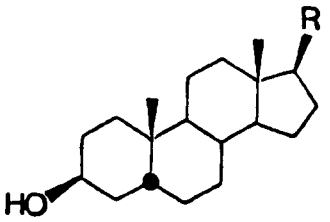
WOLLRAB V. (1969). Secondary alcohols and paraffins in the plant waxes of the family Rosaceae. Phytochemistry, **8**, 623-627.

YON D.A., J.R.MAXWELL and G. RYBACK (1982). 2,6,10-Trimethyl-7-(3-methylbutyl)-dodecane, a novel sedimentary biological marker compound. Tetrahedron Lett., **23**, 2143-2146.

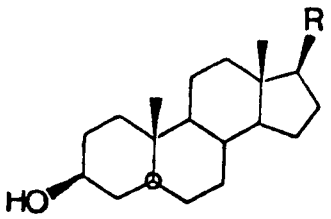
ZIELINSKI J., W.C.M.C.KOKKE, T.B.TAM HA, A.Y.L.SHU, W.L.DUAX and C. DJERASSI (1983). Isolation, partial synthesis, and structure determination of sterols with the four possible 23,24-dimethyl-substituted side chains. J. Org. Chem., **48**, 3471-3477.



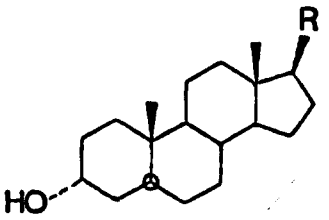
I



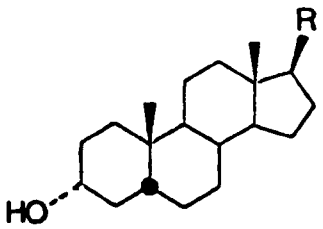
II



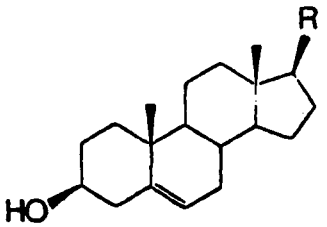
III



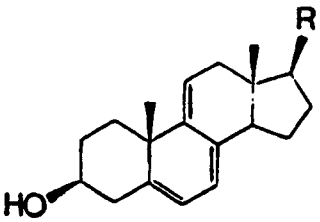
IV



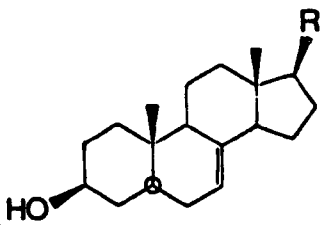
V



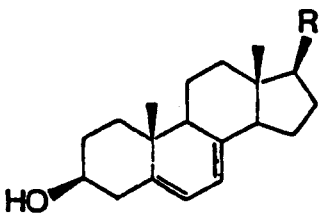
VI



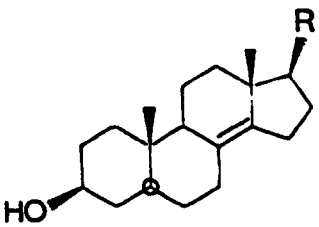
VII



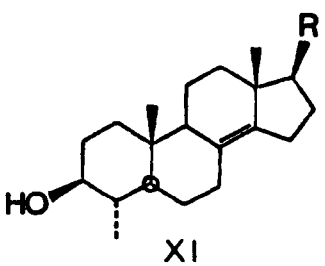
VIII



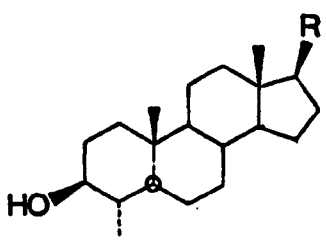
IX



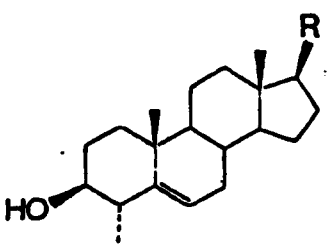
X



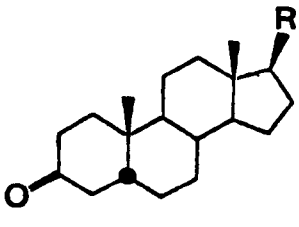
XI



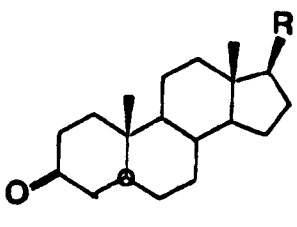
XII



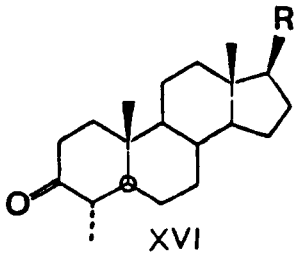
XIII



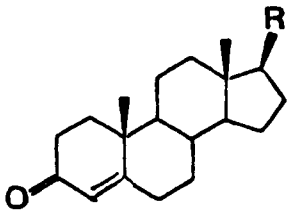
XIV



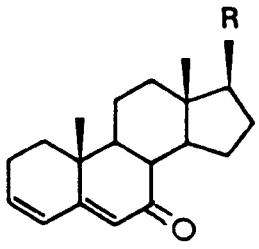
XV



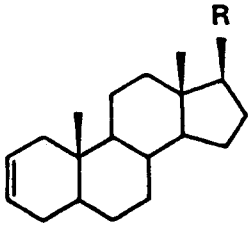
XVI



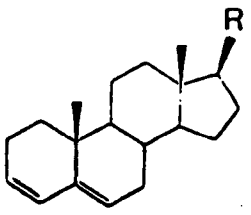
XVII



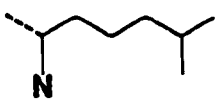
XVIII

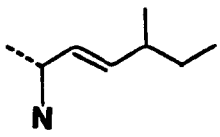


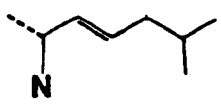
XIX

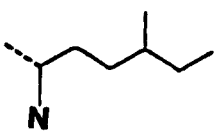


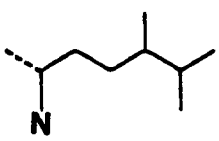
XX

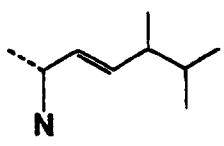
a 

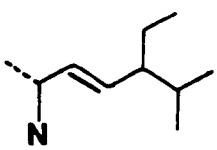
b 

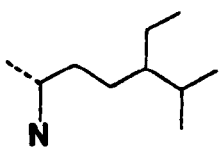
c 

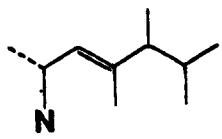
d 

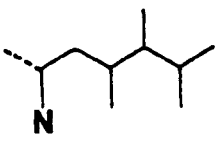
e 

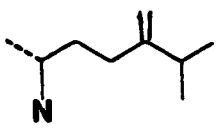
f 

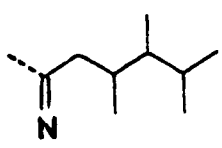
g 

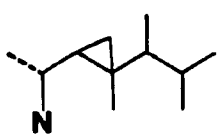
h 

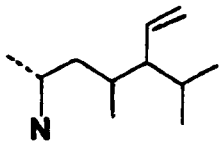
i 

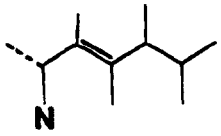
j 

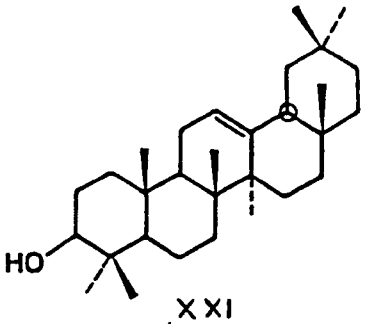
k 

l 

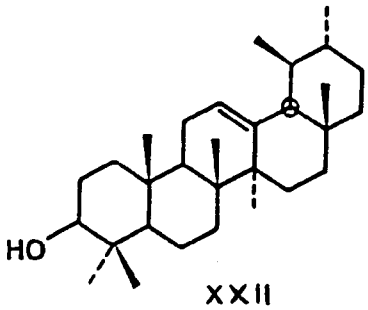
m 

n 

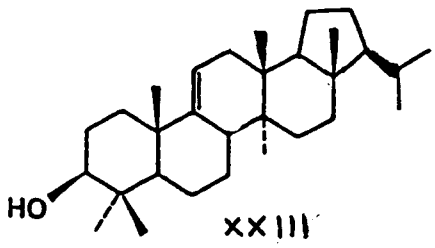
o 



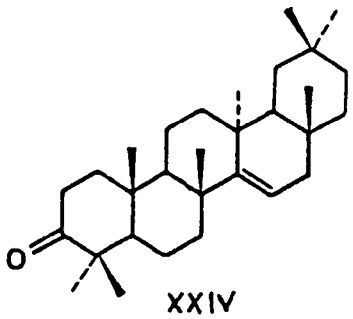
XXI



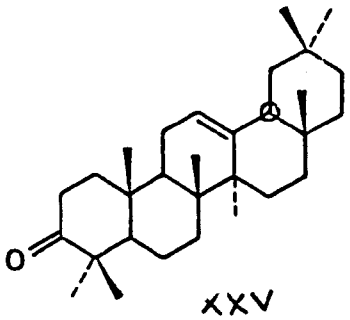
XXII



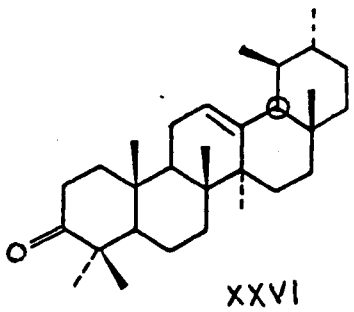
XXIII



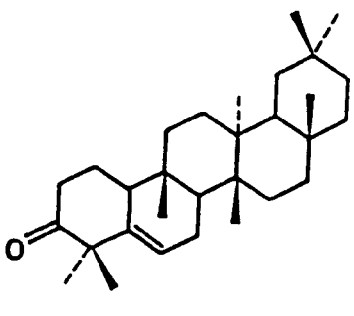
XXIV



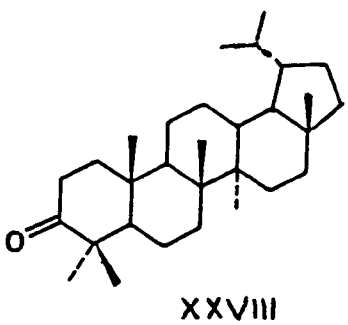
XXV



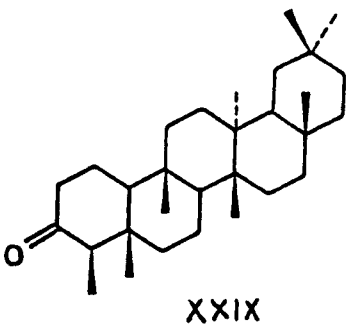
XXVI



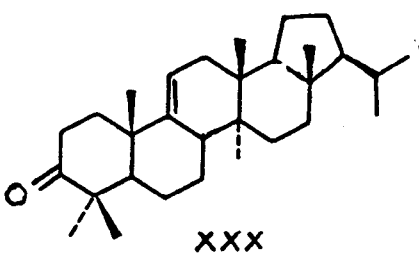
XXVII



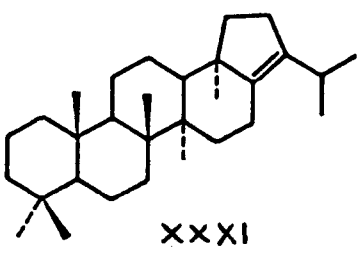
XXVIII



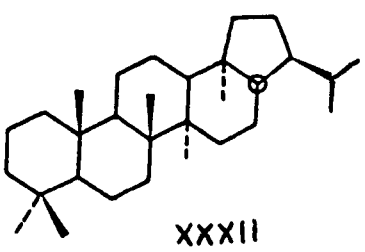
XXIX



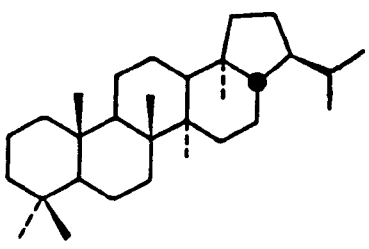
XXX



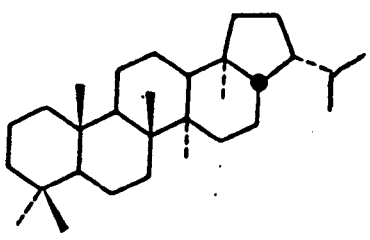
XXXI



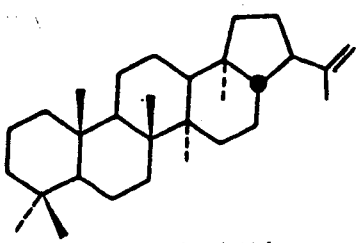
XXXII



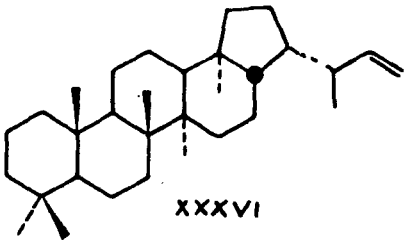
XXXIII



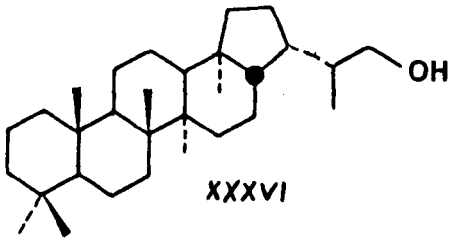
XXXIV



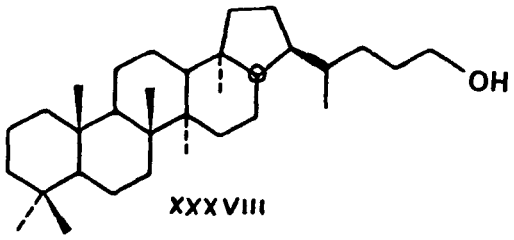
XXXV



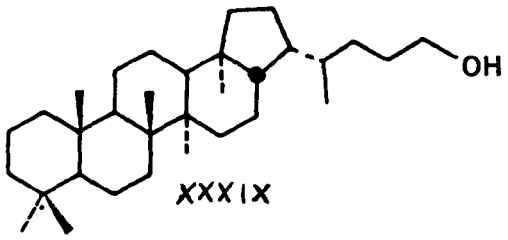
XXXVI



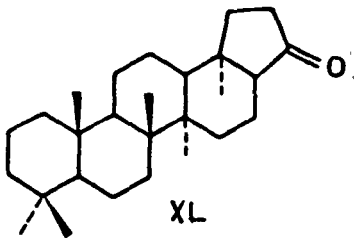
XXXVI



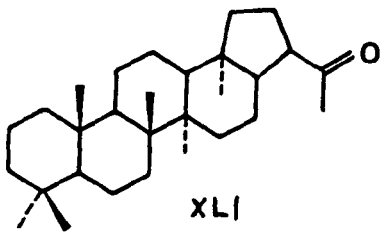
XXXVIII



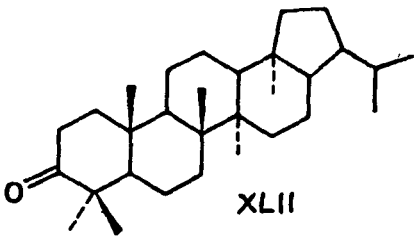
XXXIX



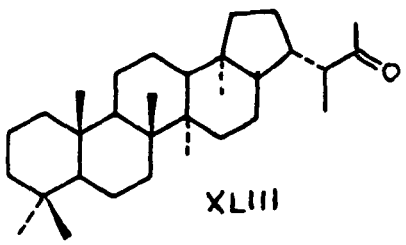
XL



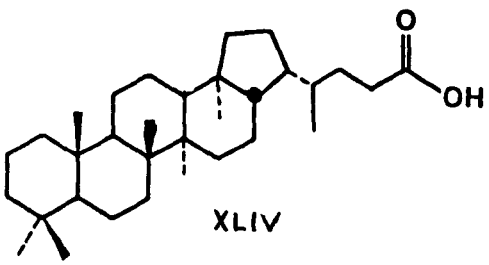
XLI



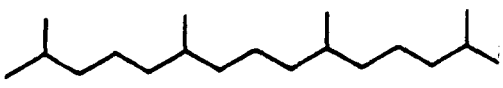
XLII



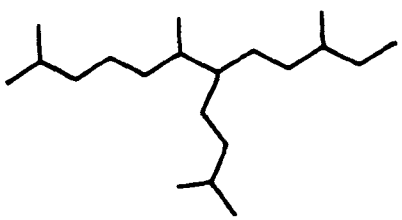
XLIII



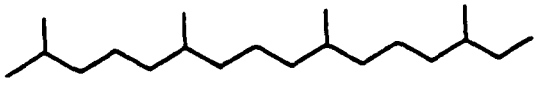
XLIV



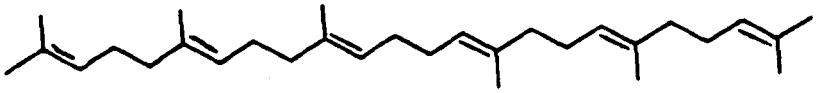
XLV



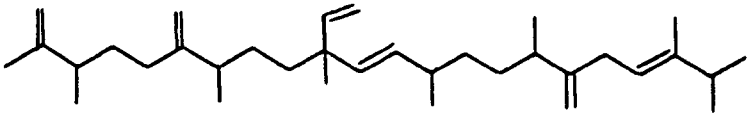
XLVII



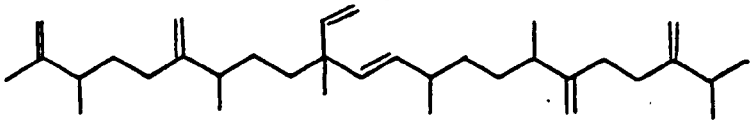
XLVI



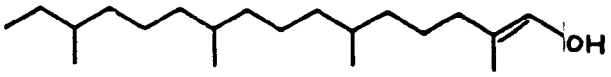
XLVIII



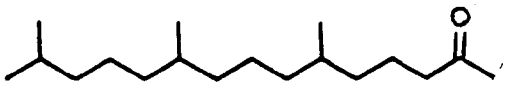
XLIX



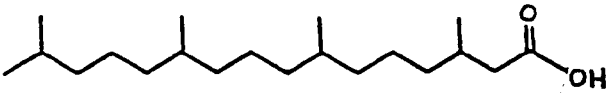
L



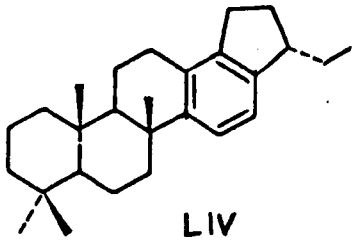
LI



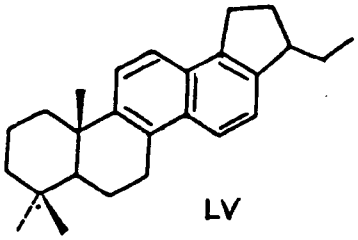
LII



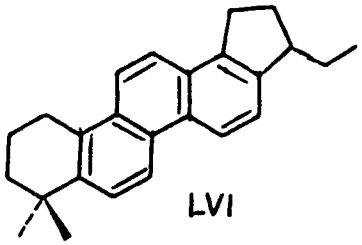
LIII



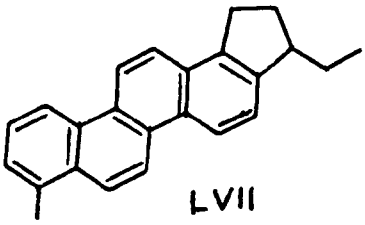
LIV



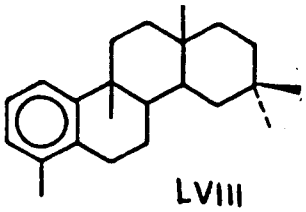
LV



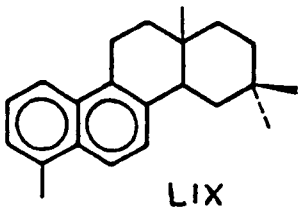
LVI



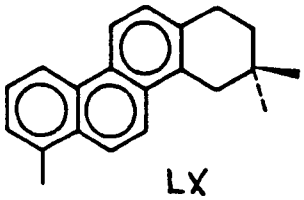
LVII



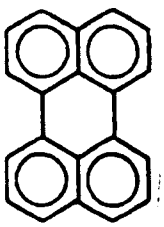
LVIII



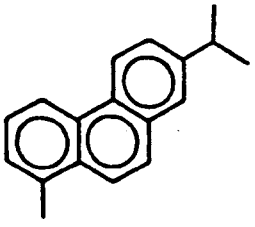
LIX



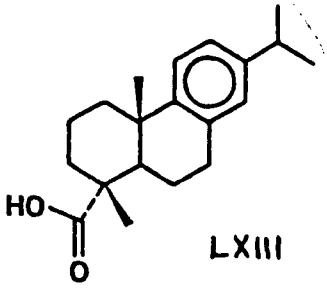
LX



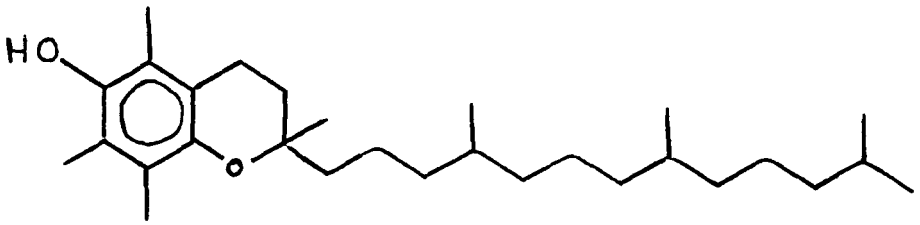
LXI



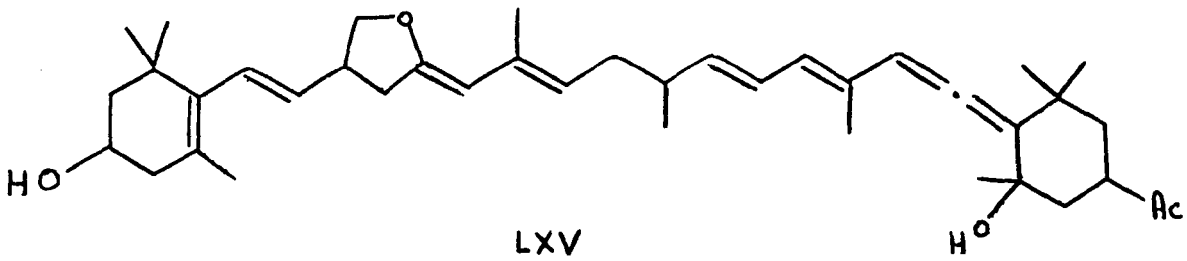
LXII



LXIII



LXIV



LXV